

PAROXYSMAL HAEMOGLOBINURIA
with special reference to its
Pathology and the production
of an Antitoxine.

Thesis for the degree of M.D.

by

JOHN EASON, M.B., C.M.



(1)

Since Dressler published the first satisfactory description of a case of Paroxysmal Albuminuria and Chromaturia in 1854, the pathology of this disease - now known by the more appropriate name, Paroxysmal Haemoglobinuria - has had devoted to it a very considerable amount of investigation, which however has not given results sufficing to explain its pathology.

The literature of Paroxysmal Haemoglobinuria is very copious and the theories regarding its pathology have been so numerous and varied that it would now require considerable space to give a complete synopsis of them. Many of these theories are speculative opinions of little or no value.

The present research is indeed not intended to be a complete historical account of already published writings, although many of those, which are of permanent value, must necessarily be alluded to whenever consideration of the pathology of the disease is entered upon.

My chief object is to describe certain experiments which I have been enabled to conduct by the methods of research introduced in quite recent years by Ehrlich

and Morgenroth. (2)

A brief description of the chief clinical features of this somewhat rare disease will be an appropriate/

appropriate preface to the considerations, upon which I am about to enter, regarding the Pathology of the disease.

The disease is one which runs a chronic course and as its name indicates, is characterised by the occasional excretion of Haemoglobin in the urine. This occurrence is determined, in many cases, by exposure of the predisposed individual to cold and, in less frequent cases, results from physical or mental exertion.

The symptoms of an approaching paroxysm are briefly as follows :- Sensation of intense cold especially in the extremities of the limbs which become pale and somewhat cyanosed, rigor follows and the temperature may rise three or four degrees. Yawning, sickness, vomiting and diarrhoea may occur and there is generally a dull pain in the lumbar region.

In the great majority of paroxysms a reddish brown or porter coloured urine containing free Haemoglobin is passed a few hours after the first symptoms have occurred.

The paroxysm may last only a few hours and the patient soon again feels as well as before, provided the precipitating cause is removed. In mild paroxysms, albumin only may appear in the urine and probably there are other insignificant disturbances when even albuminuria is absent.

Microscopic/

Microscopic examination of the urine in the typical paroxysms reveals the presence of granular pigment, some imperfect blood cells and epithelium. On spectroscopic examination the bands, characteristic of oxy- or met-haemoglobin, are to be seen.

(3)

Since the publication of Chvostek's monograph a new epoch in medical science has begun with the work of Bordet and Ehrlich on Immunity, and the initiation of the present research is due to the somewhat general knowledge I possessed of those famous researches. It occurred to me that the clinical features of Paroxysmal Haemoglobinuria were such that an investigation of the disease by the methods of Ehrlich was required; and the most careful consideration that I could give to all the circumstances of the disease encouraged me to form the opinion that the pathology of Paroxysmal Haemoglobinuria would ultimately be explained by the information thus obtained.

I began in 1903 with the working theory that we have probably to deal with a haemolysin which might or might not be composed of two active substances amboceptor* and complement**.

The following possibilities occurred to me regarding the supposed blood toxine.

(1) /

* Intermediary body, Fixateur etc.

** Alexin etc.

It might be

- (1) a toxine whose usual habitat was not the blood, but whose passage into the blood was determined by such factors as cold on the surface of the body or muscular exertion.
- (2) A toxine whose development in the blood was promoted by such factors.
- (3) A potential toxine which acted only under the influence of such factors.
- (4) A combination of two or more of these.

Considering the enormous destruction of corpuscles that occurs, the supposed toxine must have great virulence and will in consequence readily lend itself to the proposed investigation, provided one can reproduce all the necessary conditions.

Regarding these it is necessary again to emphasise as one of the greatest characteristics of the disease, the determination of paroxysms by exposure of the patient to cold or by the physical or mental efforts of the patient. ^{and} In fact ~~the~~ ^{the} relation of ~~these conditions~~ ^{cold and fatigue} to the paroxysm and the destruction of red cells during the paroxysm may be considered the most constant features of the disease.

One naturally assumes that destruction of blood corpuscles is an essential feature in all paroxysms, whether this is associated with the excretion of Haemoglobin or Albumin in the urine or in default of/

of such outward direct evidence of corpuscle destruction. Haemoglobinuria itself is not a constant symptom of the paroxysms, but its occurrence is so striking that from this the name of the disease is derived. It is to be remembered, ^{however} that haemoglobinuria may occur under numerous circumstances which may be easily differentiated from those of Paroxysmal Haemoglobinuria.

How was noted
While considering the characteristic relation of cold to the paroxysms of this disease, allusion may be made to the well established fact that Haemoglobinuria sometimes occurs as the result of excessive heat applied to the surface of the body such as occurs in some extensive burns even of a mild degree. It may be that heat initiates corpuscular destruction in an entirely different manner to that of cold in the disease now being considered, but it may be also that in both cases we have to do with the action of some toxic substance. I do not propose to consider the manner in which haemoglobinuria is brought about in cases of burns, but its occurrence and causation appear to me sufficiently analogous to prompt this passing notice. When the pathology of Paroxysmal Haemoglobinuria comes to be fully understood it may be found that the more or less severe Raynaud's symptoms, which are a/

a constant feature of the paroxysm, play an important part at these periods. The theories (5) of Murri* and of Chvostek, although they differ in important respects, are both based on the assumption that there exists in this disease a state of unusual vaso-motor irritability. Whether this be so or not the condition of the vessels of the extremities during paroxysms is one which must have an important effect on the temperature of the limb. The significance of this will be discussed later.

Paroxysmal Haemoglobinuria appears to me to possess a definite clinical resemblance to two other diseases viz - Malaria and Pernicious Anaemia; and there will be certain advantages in indicating here in what this resemblance consists.

In each disease there is a more or less frequent paroxysmal destruction of corpuscles, and intervening between the various paroxysms there are periods in which there is little or no destruction demonstrable. The paroxysm in each is heralded by a premonitory period of varying duration. There is sometimes only slight malaise or there may be a greater degree of discomfort and shiveriness, a rigor may occur of quite a severe kind, accompanied sometimes by sickness, vomiting and headache. The temperature rises and the pulse becomes more frequent. The spleen/

* Loc. cit. pages 80 and 90.

spleen may enlarge and the complexion receives a yellow "jaundiced" tinge.

The urine contains albumin. In malaria and more frequently in paroxysmal haemoglobinuria there may also be haemoglobin in the urine. In pernicious anaemia the corpuscular destruction is a less rapid process and the organism is able to transform the haemoglobin and sometimes all the corpuscular albumin as it is set free. Consequently Haemoglobinuria does not occur, Albuminuria may be absent and there is instead Urobilinuria.*

(6)

Goodall, writing of Pernicious Anaemia, adopted a view which is analogous to that of Chvostek in the case of Paroxysmal Haemoglobinuria in so far as each accepts as an essential condition, a developmental defect of the cell - a defective haemogenesis. Whether this view be correct or not, the ultimate cause of the two diseases is not thereby revealed. Indeed the theory of defective haemogenesis is not incompatible with the theory that the destruction of the cells occurs by the action of a blood toxine. The genesis of cells may surely be pathologically modified by those circumstances which are concerned in the destruction of fully developed cells.

Proceeding with the comparison of these three diseases I come to some essential points of difference/

* Wm. Hunter considers that the absence of Haemoglobinuria in Pernicious Anaemia is due to the Portal Area being the seat of the haemolysis, and to some further changes occurring in the haemoglobin. Pernicious Anaemia pages 158-166, 208, 259 & 262. 1901.

difference, recognition of which will also be an aid to the investigator of paroxysmal haemoglobinuria.

It is well known that the paroxysm of malaria is the consequence of those vital changes in Laveran's organisms occurring within, and causing the destruction of the red corpuscles of the host.

The paroxysm is frequently over in a few hours just as in Paroxysmal Haemoglobinuria. On the other hand in paroxysmal haemoglobinuria no organism has been found either within the corpuscle or contained in the plasma. In pernicious anaemia we have as a rule a much less evident paroxysm which rarely proceeds further than a feeling of malaise, moderate gastro-enteric symptoms, slight rise of temperature and increased yellow tinge of the skin. These symptoms however may continue for several days during which one assumes there is a steady but moderate destruction of the red corpuscles and then the critical period passes and improvement again takes place. The acute period is not so intense and is more prolonged than in the other diseases. It resembles Paroxysmal Haemoglobinuria in so far as the recurrences are at quite irregular periods. The Paroxysms probably result from the absorption or develop-
(7)
ment of a toxine but what induces this absorption or development is unknown.

In the case/

In the case of Paroxysmal Haemoglobinuria the defect in our knowledge is not with regard to the exciting causes which are cold and fatigue. Our ignorance is regarding the ultimate pathology and writers have hitherto been entirely at variance as to a satisfactory theory.

No intraglobular organism has been discovered such as exists in the case of malaria although it is always possible that a specific organism may be the direct or indirect cause of the changes characterising the disease. Such influences as chill are known to predispose to the development of organisms but physical or mental exertion does not usually act in this way.

X It would therefore be important to ascertain if these influences, cold and exertion, affect vital processes in a similar way the one to the other, so that some explanation may be found for their mutual etiological relation to the paroxysms. The only apparent answer is, that each has a stimulating effect on metabolic processes and on the amount of waste matter to be excreted from the blood. This aspect of the subject will be discussed hereafter in greater detail. X Another difference between paroxysmal haemoglobinuria and the other diseases with which it is now being considered, is the greater/

Insert later

X
↑

that exists greater tendency, in the former for the Haemoglobin to be excreted free and in soluble form in the urine. Sometimes Haemoglobinuria occurs in malaria but that is comparatively seldom when one considers the large destruction of red corpuscles which takes place.

This is a difference of great significance and

brings us to the question of the Pathology.
 meantime I will assume what I hope later to prove

that the Haemoglobin is apparently set free in a soluble state in the blood of those suffering from Peroxysmal Haemoglobinuria. Its discharge into

the urine is thus facilitated. In malaria on the other hand we have to do with a mechanical fracture of the cell, the pigment in a granular form being contained partly in the spores and partly in the plasma. Haemoglobinaemia in the true sense is therefore not the rule although pigment in an undissolved form is present in the plasma.

Haemoglobin in this undissolved state will not pass easily into the urine but will be caught and retained in the various tissues of the body. In any case there is the difference to note between these two diseases, *former* that in the *one* a large destruction of corpuscles takes place without escape of Haemoglobin as such from the body, while in the *latter* other a large destruction of corpuscles is followed by the passage of the Haemoglobin into the urine. It is improbable that this difference depends on the relative amount of corpuscular/

Malaria & P.H.

corpuscular destruction characteristic of the two diseases, but is rather one which is dependent on the nature of the change produced in the Haemoglobin.

The occasional occurrence of Haemoglobinuria in malaria is probably not entirely explained by the action of the organism, but the haemoglobin thus freed from the corpuscles may then be dissolved by the action of some additional toxic substance in the serum.

(9)

Grawitz, in a paper on blood poisons, states that there is (A) a granular degeneration of cells not associated with Haemoglobinuria (B) a plasmolytic change in the blood causing haemolysis and haemoglobinuria. This seems to constitute the difference that exists between these two diseases in regard to the occurrence of haemoglobinuria. In all three diseases ^{how under comparison} however haemoglobinaemia has been found in some degree but apparently in only one of the diseases viz - paroxysmal haemoglobinuria does the plasma attain the haemoglobin saturation which Camus ^{(9) & Ponfick (10)} stipulates as necessary for the production of haemoglobinuria.

Camus has made most careful experiments on the dog from which he concluded that 0.230% haemoglobin must be free in the plasma before haemoglobinuria can occur. He found that the destruction of 75,000 red corpuscles per c.m.m. or $\frac{1}{57}$ of the total was necessary in order to produce the above concentration of free haemoglobin in the plasma. This figure is well within/

within the limits of possible error in estimating the corpuscles.

I can only add that Camus' experiments appear to me to have been conducted in such a way as to make his conclusions very reliable.

(10)
Ponfick had already many years ago demonstrated that $\frac{1}{60}$ of the blood corpuscles requires to be destroyed in order to produce Haemoglobinuria.

(11)
Limbeck has said that "the fate of Haemoglobin dissolved in the plasma depends on the degree of activity of the liver cells which tend to change the Haemoglobin into other pigments. We get Haemoglobinaemia and Haemoglobinuria if the capacity of the liver is exceeded" in the direction already indicated. "Haemoglobinuria therefore indicates a very considerable destruction of red corpuscles" or, as a logical inference from Limbeck's statement, a diminished capacity of the liver to perform this function attributed to it by this author.

(12)
Camus has done experiments on animals with the object of determining the power of the liver to check Haemoglobinuria. In the dog or rabbit, sacrificed by bleeding, and submitted to lavage of the liver, he found that a haemoglobin solution could/

could be injected into the portal vein and collected afterwards still exhibiting the original depth of colour.

Repeated injections of the fluid ultimately rendered it paler but the amount of haemoglobin arrested was not of such amount as could prevent the occurrence of haemoglobinuria. A like result was also obtained by injections into the spleen.

(pages 36 - 39, 45) Limbeck's statement regarding the relation of the liver to haemoglobinuria emphasises the need there is to have the state of the metabolism, during the paroxysm, investigated.

Camus' experiments however made it appear that the liver does not possess the function attributed to it by Limbeck. Moreover it has lately been shown that the intervention of the liver is not required in order that Haemoglobin may be transformed into Haematoidin. This may result from the action of the serum (Maragliano).
(13)

The analogy between Paroxysmal Haemoglobinuria on the one hand and Malaria and Pernicious Anaemia on the other, and the distinctive features of each have been stated at some length. My object in doing so has been that I might indicate the ideas, which influenced me 3 years ago to begin this research.

(14)
Luzzatti and Sorgente have also suggested an analogy viz between the febrile attacks of Pseudo-lukaemia./

lukaemia and splenomegaly on the one hand, and those in Paroxysmal Haemoglobinuria on the other hand. By the sudden diminution of the diseased spleen in the former conditions these authors assume that there is injected into the circulation certain pyrogenous substances, while in Paroxysmal Haemoglobinuria, cold acts either by hindering the excretion of toxic substances or by favoring the passage of these substances from the seat of origin into the general circulation, where haemolysis would be caused.

They say that the latter possibility has to be excluded because in the ligatured arm of an individual affected with this disease the conditions are not favourable to the passage of the toxine into the general circulation, but nevertheless, local destruction of corpuscles is the sequel. This latter statement must surely be qualified, for are we not rather bound to accept that the blood in stasis (whether artificially or otherwise produced) must come to contain a higher percentage of the physiological or pathological products of metabolism?

The experiments which I shall describe hereafter will demonstrate what significance, if any, is to be attached to the analogy of Luzzatti and Sorgente.

Before/

Before concluding these general remarks mention
(15)
may be made of Boas' classical experiment in which
he ligatured a finger of his patient who then kept
it immersed in cold water for some minutes. As a
consequence the blood of the finger became Haemo-
globinaemic. Luzzatti and Sorgente have concluded
from this that the necessary conditions for the
production of Haemoglobinaemia do not appear to be
directly dependent on, and are perhaps in no way
related to the activities of the central organs, so
far as the production of haemoglobinaemia is
concerned. There is possible fallacy here. The
central organs may be concerned in the production
of a substance which enters the blood. This
substance may possess potential toxicity, which is
manifested only under the influence of some factor
such as cold. Boas observed further that as a
result of the complete immersion of the body in
cold water a general disturbance accompanied by
haemoglobinaemia and haemoglobinuria ensued.

The theory that this disease is consequent on
the presence of a toxic substance in the blood, was
(16)
first suggested by Wiltshire in 1870 and, in the
time that has since elapsed, occasional opinions
have been expressed in support of such a theory.
Wiltshire's view was, that the "disintegrating agent"
was/

was a volatile organic acid, probably a fatty acid which was thrown back into the system by chilling of the skin and thereby "auto-genetic poisoning" occurred.

(17)

Ilgner considered it an infectious disease.

His patient had Malaria previously. Ehrlich's

(18)

original view was that a proportion of the red cells were hypersensitive to the action of cold.

He maintained that this was proved by ligaturing and immersing a finger in ice-cold water for $\frac{1}{4}$ hour and then in tepid water for $\frac{1}{4}$ hour. The serum

(19)

was then haemoglobinaemic. In a later publication

he found he could not confirm this in vivo

experiment by a somewhat similar one in vitro.

He then concluded that the vessel endothelium under the influence of cold, produces substances (ferment) which destroy the corpuscles.

(20)

Charpentier thought that under the influence of cold combined with malarial infection there was produced a toxine which could disintegrate the corpuscles directly or by indirect action through the vaso-motor centre. His views were not supported by any experimental investigation.

(21)

Prior investigated the literature and conducted much work to which reference will be made later. He formed the opinion that a toxic substance/

substance originates in the alimentary canal and passes into the blood circulation. Such a view has some resemblance to that of Hunter in the case of Pernicious Anaemia. Chvostek considers the formation of toxic material by the vessel endothelium at least improbable because destruction of corpuscles takes place in vitro by shaking. (Loc.cit.page 65) An abnormal property of the serum cannot be demonstrated (pages 69-70)

Hayem in 1889 indicated that there was probably a toxaemic condition of the blood in this disease.
(22)
He has recently given his views on this subject in his published lectures. The paroxysm is considered to be due to a toxaemia in individuals affected with a chronic alteration of the blood. Assuming this change in the blood, he considers that the paroxysm can then be determined by causes which increase the waste products circulating in the blood, such as cold and fatigue. He refers to the syphilitic origin of many of the cases and believes that this accounts for the blood in these cases being rendered vulnerable, just as in paludism the blood is rendered vulnerable to quinine. In the absence of syphilis the corpuscles may be rendered vulnerable by other causes such as gastritis of alcoholic origin which was present in one of his cases.

In/

In short his view is that cold or fatigue produce toxic substances which, while they do not cause the destruction of normal cells, are yet sufficient to destroy the more vulnerable cells of individuals, predisposed by a certain toxæmic state of the blood. His chief reason for accepting a toxic origin is the presence of Methaemoglobin in the urine, and he considers the corpuscle destruction takes place in the kidney, where alone the toxic substances would be present in sufficient concentration to produce this change.

(23)

Luzzatti and Sorgente think that the blood serum during the paroxysm has a haemolytic action. The serum of the interval has no abnormal or strong haemolytic action but the serum of the stasis blood produces quick haemolysis unless the part affected by stasis is kept warm. If the ligatured limb is kept warm the serum separated from the blood is much clearer than if the unligatured limb is subjected to chilling before taking away blood. This experiment minimises the intrinsic importance attributed to the action of stasis by Chvostek.

Luzzatti and Sorgente, agreeing with Hayem, believe that serum which separates coloured by/

by Haemoglobin has no further haemolytic action. After the paroxysm the serum resumes its normal conserving power.

Contrary to the view of Hayem they believe that destruction takes place in the peripheral vessels and not in the internal organs as a rule. They do not deny that there may occasionally be cases of renal origin.

Blood obtained from their patient in the interval gave up a clear serum (a) after coagulation at the room temperature or on ice (b) by centrifuging. But if the temperature was changed from that of ice to 37°C a rose red serum was sometimes obtained.

To this they attached no importance as by the same means a coloured serum was obtained from the blood of healthy children. After many experiments in vivo and in vitro they concluded that cold produces certain changes within the organism which cannot be produced by this agent in vitro.

In regard to the toxic theory of Paroxysmal Haemoglobinuria the foregoing opinions are those which had already been published when an opportunity presented itself to me, to study two cases of this disease which came under my observation at the out-patient Department of Leith Hospital.

After full consideration of the chief features of/
 of/

of the disease, and keeping in view certain facts and ideas regarding Malaria and Pernicious Anaemia, I became eager to know if in the case of Paroxysmal Haemoglobinuria we had to do with the action of a haemolytic body, and if so, then to ascertain if this body produced its action in the same way as Ehrlich and Morgenroth have maintained for the immune sera viz by means of amboceptor and complement.

The following are brief histories of my cases.

Notes from case book (1901)

T.S. aged 38, came to the New Town Dispensary, Edinburgh in the autumn of 1901. I obtained leave from Dr. Wm. Elder to have him under observation in Leith Hospital where he was admitted on November 18th. He complained of "Tremblings of cold and pain in the stomach."

Family History. Father died at the age of 50, cause unknown. Mother died, aged 60, of "swelling of the face and head." He was married three years ago (1898) and has no children. So far as he knows his wife has had no miscarriages. He worked as a twine spinner, a sailor, a fireman, and latterly as a /

a tank and ship painter. He has sailed to the Baltic but he had never contracted any fever on his voyages.

Previous Health. Has had scarlet fever, small pox, rheumatism.- All in childhood. He was six weeks in Hospital suffering from a bruise of the back when 17 years of age. He was in the Edinburgh Royal Infirmary, Lock ward for three months when aged 27. There was no rash or sore throat. Two years ago (October 1899) when he was cleaning and painting tanks he had a violent shiver one morning, his toes and fingers becoming as if frost bitten. He sheltered in the bothy but although there were two fires there he could not get warm. He vomited several times. After three hours he went home, had hot gruel and began to feel better. He noticed that the urine he passed during the afternoon, was dark reddish brown in colour. He felt much better next day, but greatly exhausted. His urine was clear again. He went to the Out-patient department at Leith Hospital and got some medicine but did not return. He remained well till March 1900 when he had a similar attack. During the summer of 1900 he remained well but in the following winter he had attacks every month or two until lately./

lately. They then began to recur every time he exposed himself to cold, sometimes daily.

He had never suffered from numbness of the toes and fingers until his first attack two years ago. He suffers slightly from constipation, an aperient being required occasionally. He has suffered from pain in the stomach for about two years. The pain is sometimes griping in character and comes after taking food. This may last for a few days and then it may go away for a fortnight.

He frequently suffers from vertigo. He has also pain in the cardiac region during or after an attack or after exertion.

For about three years he has occasionally suffered from shooting pains in the legs. Six months ago he completely lost the power in his legs for two or three hours.

At the time of admission in November 1901 the symptoms of the paroxysms are somewhat similar. In addition to what has already been stated, prominent features are, great thirst, pain in the lumbar regions posteriorly and in right lumbar region anteriorly, but not on left side. His skin becomes "jaundiced." The attacks are not so frequent in hot as in cold weather. The dark urine/

on the urine passed a day before admission, was examined spectroscopically. The two bands of oxy-haemoglobin in the green-yellow part of the spectrum were present. On the addition of ferricyanide of potash the two bands became fainter and a third band in the red appeared (methaemoglobin).

Very few corpuscles were found on microscopic examination of the urine which was exceedingly dark, with a heavy brown deposit.

Condition on admission. Patient has a sallow complexion and somewhat dull expression. His lips and conjunctiva are pale. Teeth are very bad and a blue line along the edge of the gums is well marked. He is fairly well nourished but says he has been getting much thinner during the last three years. ^{years ago} Three ^{years ago} he weighed 9 st. 4½ lbs. and now he is 6 st. 9½ lbs.

Alimentary System. In addition to the symptoms already described, he occasionally suffers from flatulence, eructations and waterbrash. On palpation of abdomen there is slightly increased resistance felt at the left edge of the rectus; some tenderness also elicited on deep palpation in the same region: lower border of stomach and liver normal. The condition of the Circulatory and Respiratory Systems is good.

Haemopoietic/

Haemopoietic System. No enlarged glands palpable.

Spleen normal size.

Blood: Haemoglobin 40%

R. B. C. 3,720,000

W. B. C. 8,700.

Urinary System. Urine as before described during paroxysm. Urine of Interval. Straw coloured, some floating mucus. Reaction acid.

Specific gravity 1010. No albumin, sugar or bile.

Nervous System. Knee jerks slightly exaggerated.

November 27th. Hands and feet were immersed in cold water at 6 a.m. for 20 minutes. He began to feel chilly, and at 8 o'clock he was visibly shivering, had nausea, and could not take breakfast. At 9 a.m. had a rigor. He passed a small quantity of urine, dark in colour, containing albumin and giving the blood reaction with guaiac and ozonic aether. Microscopical examination showed pigmented debris, but no red cells

December 8th. A paroxysm was artificially produced as on November 27th. During the rigor he had a bad cough and had pain between the shoulder blades.

The temperature rose to 102.4°F. The urine, passed twelve hours after the first specimen of haemoglobin-uric, urine, was almost free from all trace of albumin/

albumin and had again a clear straw colour.

Immediately before the paroxysm the following facts were ascertained regarding the blood.

<u>R. B. C.</u>	<u>3,175,000</u>
<u>W. B. C.</u>	<u>20,620</u>
Haemoglobin	44%

Immediately after paroxysm

R. B. C.	3,050,000
<u>W. B. C.</u>	<u>15,300</u>
Haemoglobin	40%

February 5th. A paroxysm was produced again to-day by immersing the patients feet in cold water. A careful chemical examination of the urine was made on this and the former occasion. The results of this analysis will be found on page, 136. The patient was discharged on February 12th 1902, having gained 8 lbs weight. He had no paroxysms except those artificially produced.

He has been three times in Leith Hospital since but there is practically nothing new to record. He has continued to have the same susceptibility to cold. I have learned from him that while in the Lock ward he had a chancre.

CASE II. M.R. a girl 6½ years of age admitted to Leith Hospital on December 18th 1902 complaining of/

of "attacks of weakness and red urine." Duration 1 year and 9 months.

Family History. Parents both living and healthy. The mother has frequently suffered from bloodlessness and had bleedings from the nose when a girl. There is one sister who is healthy.

Personal History. Patient used to be punished by whipping pretty frequently and the mother is inclined to think that this may have caused the present trouble. The conditions of the home are not very satisfactory. The father is a labourer and known to me to be very lazy and frequently idle. The mother has to go out and work as a charwoman.* There are four members of the family who live in a one roomed house. Until the present illness M.R. has been a stout strong child. She had measles when 2 years old.

Present Illness. One day in September 1901 Mrs. R. noticed that the urine passed by her child was "bright red" in colour. The child had not been exposed to cold at the time and the mother does not know of anything that could have caused the ^{illness.} These attacks continued for 6 months, lasting for a day or two and stopping entirely for two or three days at a time. The attacks were always more severe when the weather was cold.

At this period of the illness her appetite and/

* The mother had four successive miscarriages following the birth of M.R.

and state of nourishment was well maintained. The attacks were however getting worse and she was taken to the Out-patient department at Leith Hospital and she was admitted on December 11th 1901. For a week before admission, all the urine passed was dark brown and at the time of admission it gave blood, albumin, and pus reactions. After admission at this time she passed clear straw coloured urine in which no abnormal constituent was found. She was then pale, apathetic and had a harsh dry cough.

Blood condition. R. B. C. 3,475,000

W. B. C. 6,170

Haemoglobin 50%

Other Systems normal.

She left hospital on January 5th 1902 apparently cured (sic) but in 3 or 4 days she was again as bad as ever. She was taken to the Children's Hospital, Edinburgh where she remained seven weeks but was discharged in statu quo, measles having broken out in the ward. The diagnosis, on each occasion she was in Hospital, was Paroxysmal Haemoglobinuria.

Since July 1902 she has been under my observation and had attacks of Haemoglobinuria every now and then. Mrs. R. says she always knew when her child was about to pass red water as this was/

was preceded by the complaint of feeling cold which caused her to crouch as near the fire as possible and "to pile on coals whenever her parents' backs were turned." She also complained at these times of headache, and there was occasionally some sickness.

State on admission. A pale, anaemic rather listless child irritable and easily set crying.

Heart nothing abnormal.

Respiratory System. Has a cough which gives no pain.

A few rhonchi but otherwise nothing unusual noted.

Urinary System. No abnormal constituents, although patient was bathed on admission and the temperature rose to 103.4° F. Previous to the bath the temperature was 98.6.

She was discharged on January 13th 1903 having had no paroxysms in Hospital. An examination of the urine was made daily without abnormal constituents being found.

I have since had many opportunities of confirming the diagnosis of Paroxysmal Haemoglobinuria in M.R. I have frequently obtained specimens of dark porter coloured urine, containing much Haemoglobin but few or no red cells.

Technique. This varied to an extent which will be defined as I proceed with the description of the various experiments. At first, it was desirable to ascertain by the simplest methods, if there were sufficient reasons for proceeding with a lengthy investigation in which the more elaborate experimental technique of Ehrlich could be employed.

My reason for proceeding thus was that I anticipated difficulties in obtaining sufficient material for a protracted series of experiments. Cases of Paroxysmal Haemoglobinuria are so rare that experiments may suddenly be brought to an abrupt termination.

That this is a very common occurrence may be learned on perusing the literature of this disease. It was important therefore to cause a minimum of inconvenience to the individuals from whom I wished to obtain serum. On that account the plan of using serum, obtained from cantharides blisters* appeared worthy of trial.^(23a) There was the advantage moreover that a large amount of serum could thus be readily obtained. From a blister $2\frac{1}{2}$ inches square 10 c.c. of serum are usually available. Previous to the application of the cantharides plaster, the skin was carefully cleansed with lysol and water, then/

* 23^a is a reference to an investigation in which this method was employed, although not in Paroxysmal Haemoglobinuria.

then with methylated spirit and finally with boiled water. Before opening the blister its surface was again very carefully washed with boiled water. A Graefe knife and scissors were sterilized in boiling water and a test tube was washed, dried and plugged with wool and finally kept in an oven for two hours at 160°C . A small puncture was then made in the most dependent part of the blister and the serum was received directly into the tube, which was again closed with the sterile plug.

I thus obtained ⁱⁿ a very simple way a large amount of serum. In order to test the toxic properties of the serum on corpuscles of the same or another individual, the manipulation was again simple and therefore conducive to asepticity. One c.c. of the serum was drawn up into a sterilized pipette and was then received into a test tube already sterilized. A finger of the individual, who was to supply the blood, was carefully cleansed with lysol solution and then with boiled water and dried with wool. A surgical needle sterilized in boiling water was used to prick the finger and three drops of blood were allowed to fall into the serum. The woollen plug, sterilized with the tube, was then replaced and the serum and blood were gently shaken.

In/

In the earlier experiments the tubes were allowed to stand at the room temperature for 24 hours. It will be noted that the whole blood was used. (not corpuscles which were separated and washed from the serum.) Several very careful workers have likewise used whole blood as for instance Polk, ⁽²⁴⁾ in a recent research on the heterolytic action of human serum on rabbits blood. Polk considered his results in no way vitiated by doing so. This technique was also quite satisfactory for my preliminary experiments.

In July 1903 the patient T.S. was in hospital and had many paroxysms. He was certain to have a paroxysm if he remained up out of bed for an hour or two.

On August 10th T.S. was allowed up for two hours. He began to shiver and the characteristic symptoms of a paroxysm followed. A blister was applied to the abdomen and the serum was obtained on the following day. (Serum A.) Serum was also obtained from a man who had just recovered from Acute Rheumatism (Serum B.) and a girl suffering from nephritis. (Serum C.)

- I. Blood was obtained from the man who gave serum B.
- II. Blood was " " Resident Physician
- III. " " " myself.

1 c.c./

1 c.c.	Serum A +	Blood I	3 drops	} Serum had a deep carmine colour.
1 c.c.	" A + "	II	3 drops	
1 c.c.	" A + "	III	3 drops	
1 c.c.	" B + "	III	3 drops	serum remained "very pale."
1 c.c.	" C + "	III	3 drops	serum "very slightly tinted"

The clot which formed in the tubes varied thus

Blood mixed with Serum C gave a "tough and heavy" clot.
 " " " " B " " "dense and heavy" clot.
 " " " " A " " much less dense clot than in B and C.

Microscopical examination of the corpuscles after 90 minutes in the tube disclosed variation in the size of the cells acted on by serum A. There was no tendency to rouleaux formation. After 24 hours there was considerable variation in the size of the cells. The small cells were much crenated and deeply coloured. Other cells were large and pale.

With the microscope, the serum could also be observed to have a deeper colour. Cells, acted on by serum B, were grouped together after 90 minutes. Variation in size also present. After 24 hours the cells, besides showing grouping, were almost without exception small and their colour was uniformly deep. Not much crenation.

Serum/

Serum A therefore appeared to have the property of injuriously acting on the red cells and also that of modifying the fibrin formation. This serum could not accurately be called the serum of a paroxysm, as the blister was only applied 5 hours after symptoms began and the serum was not obtained till the following day.

Similar experiments were done on August 12th and again on August 16th when there could be no doubt that it was interval-serum.

The following refers to the latter date.

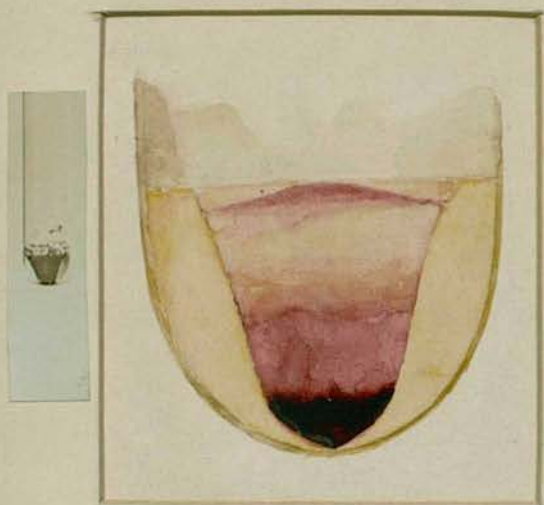
- I. Normal serum + normal blood. After 90 minutes the coagulation of the blood was complete and the serum was not coloured.
- II. Haemoglobinuria serum + normal blood. The clot was ill-defined and the serum was much coloured.
- III. Normal serum + haemoglobinuria blood.
Coagulation as in one except that some cells were agglutinated at foot of tube. The serum was not coloured.

Microscopic examination showed similar changes to those already recorded.

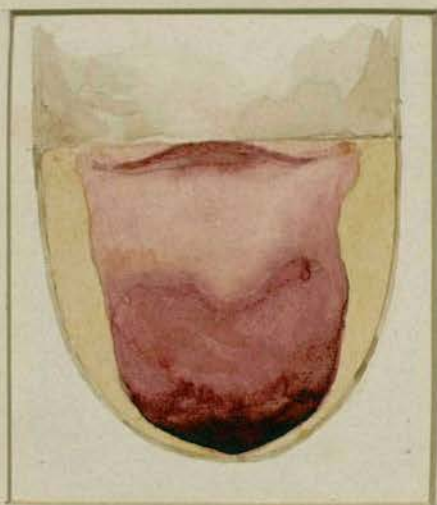
Cells in III show agglutination.

The haemolysis recorded in II occurred very early/

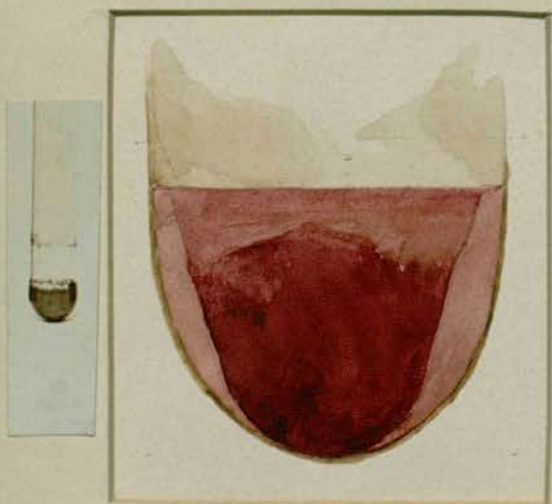
I.



II.



III.



IV.



early - 90 minutes. In subsequent experiments I found considerable variation in the time required for haemolysis by different specimens of serum.

August 23rd. In order that the serum of the paroxysmal period might be tested I availed myself of the patient's predisposition to paroxysms, if allowed out of bed.

Accordingly a blister was applied at 5 a.m.
and the patient was allowed out of bed at 10 a.m.
At 2.45 p.m. he began to be shivery. At 3.15 p.m.
he passed dark urine containing haemoglobin.

Meantime the blister had been forming and was likewise evacuated at 3.15 p.m. The serum was golden in colour and a spectroscopic examination shewed the bands of oxy-haemoglobin. There was however so little haemoglobin present that the naked eye appearance did not suggest its presence. There was absolutely no reddish tint. The following experiments were done.

- | | | | |
|----|------------------------------------|--------------|--------------------------|
| 1. | 1 c.c. normal serum + normal blood | 3 drops | <u>No haemolysis.</u> |
| 2. | " " + P.H. | " (interval) | <u>No haemolysis</u> |
| 3. | P. H. " + normal | " 3 drops | <u>haemolysis.</u> |
| 4. | P.H. " + P.H. | " (interval) | <u>Great haemolysis.</u> |

The drawings and photographs represent very well the results given in the table. In the photographs/

photographs of No. 1 and 2 the clear serum is seen at the sides with a clot in the centre. The clot was well formed and contracted.

In photographs of 3 and 4 the serum at the sides is very dark and the central clot appears large because relatively much less contracted. The clot in 3 and 4 was light and fragile. Evidently the serum of the paroxysm had a similar action to that of the interval. On this occasion the mixtures were shaken up somewhat violently and therefore the negative result in 2 speaks strongly against Chvostek's Theory.

Chvostek observed that the serum became haemoglobinaemic if the blood of his patient was put in a centrifuge which did not move smoothly and the same occurred in the serum of blood obtained by wet cupping. These observations lead him to conclude, that the corpuscles were very susceptible to mechanical injury and that haemoglobinaemia resulted from this cause (p.60-61). My last experiments indicate that Chvostek's Theory is at least not an entirely satisfactory one. The corpuscles in tube 2 withstood a considerable degree of violent shaking.

(25)

Donath has recently reported on the resisting power of the red cells in this disease after a careful/

careful investigation and he has found that the corpuscles of Paroxysmal Haemoglobinuria blood were always less resisting (in three cases) than those of normal blood. Full consideration of this aspect of the subject is meantime reserved. The results I have recorded however distinctly indicate that there is some other explanation of the haemolysis than that of Chvostek. I hope to show that Donath's results are not inconsistent with my view. I am moreover convinced from the experiments on August 10th 12th and 16th that mechanical injury is by no means a necessary condition, for the occurrence of Haemolysis.

The method of using blisters to test the serum of the paroxysmal period was very satisfactory as blood serum obtained at this period is generally so much coloured that reliable results cannot easily be obtained. Whether the toxicity of the serum of the paroxysmal period is diminished by the withdrawal of toxine which is supposed to become anchored to the red cells, ^{however} cannot be very accurately ascertained by the employment of blister serum.

The following microscopical examination was made. A drop of T.S.'s serum was placed on a glass slide along with a drop of normal blood and a cover slip was then applied. A similar preparation was/

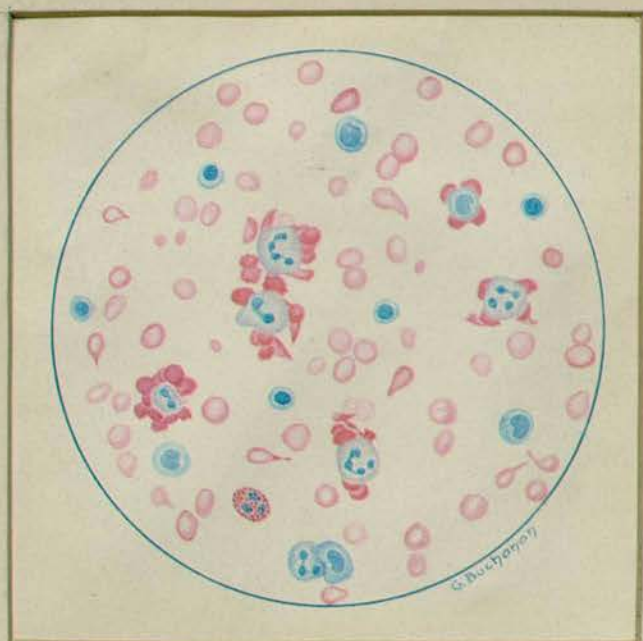
was made with the control serum which in the last tube experiment was found to possess no haemolytic action on normal blood. The two slides were then laid on a sheet of moistened blotting paper which was placed under a bell jar for one hour. The following characteristics were then noted on microscopical examination.

- Slide I.
- (1) No rouleaux formation.
 - (2) Variation in size of cells
 - (3) Poikilocytosis fairly marked. The cells assuming a lenticular shape.
 - (4) There also appeared to be a change in the condition of the pigment, which now appeared pink.
 - (5) Practically no crenation.
- Slide II.
- (1) General formation of rouleaux.
 - (2) No variation in size of cells
 - (3) No Poikilocytosis.
 - (4) No pink appearance of cells.
 - (5) Crenation distinct.

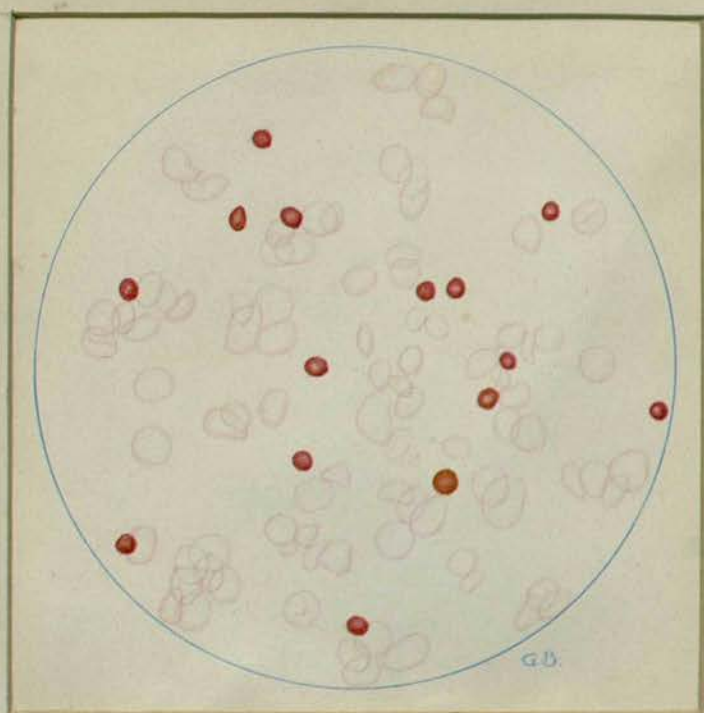
Hereafter (pages 48&49) I record a somewhat similar experiment with the blood serum of T.S.

On August 30th (during a paroxysm) blister serum was again obtained and its haemolytic action on normal/

I



II



normal blood corpuscles (my own) was again noted. A careful microscopical examination of the cells was made after the tube had been allowed to stand for 60 minutes at the room temperature. Their condition is fairly well indicated in the drawing, made for me, by Mr. Buchanan of Edinburgh University, Pathological Department. Already after 60 minutes many changes have become evident in the cells. In the size and colour of the red cells there is considerable variation, while some may be considered normal in these respects. There are many large cells which are very pale and there are others which have become contracted and possess a uniform super-normal colour. Changes in the condition of the haemoglobin within the cells are also present. In numerous cells globules of dissolved haemoglobin may be seen dotted throughout the cell. Some of these globules I have seen lying free in the serum, apparently so recently extruded from the cell that sufficient time had not elapsed in order to permit an intimate mixture of the Haemoglobin with the serum.

In a camera lucida drawing by myself (page 41) a slightly later stage of the process represented in figure 1, is to be seen. And a still later stage is shown in the figure 2 opposite. These changes/

changes may also be seen in the microscopic specimens Nos.1 & 2. The 'shadow' cells are beautifully seen in slide 2 ..

I wish to emphasise that the above description refers to the action of Paroxysmal Haemoglobinuria serum on normal cells (Isolysis) which were originally of uniform size.

(26)

Gruber's description and explanation of the process of the solution of animal corpuscles by an immune serum appears quite appropriate here. "The cells swell up as if by water and the haemoglobin is discharged. The resistance of the osmotic membrane of the rind of the cell is removed, yielding takes place and water and salts enter and the haemoglobin and other molecules go out. So far as the haemoglobin is concerned it is wrong to speak of a digestion as the haemoglobin is liberated from the cells as such. It is rather an osmosis."

I also made a most interesting observation which jointly concerns the leucocytes and erythrocytes. I attach considerable importance to the observation for reasons which I will now submit.

(27)

Levaditi by his investigations in the domain of/

of Immunity was one of the foremost to draw attention to the extraordinary activity with which phagocytes attack red cells which have become loaded with Intermediary Body. He found that the red cells when in this condition are very eagerly absorbed by the phagocytes in whose interior solution then occurs.

This appears to be an extreme degree of of the process already observed by Metchnikoff.*

Metchnikoff says :-

"Ce processus se fait d'une façon très particulière, et mérite à un haut point notre attention. Les mononucléaires poussant des prolongements protoplasmiques nombreux et très fins à l'aide desquels ils accrochent une ou plusieurs hématies à la fois. A la suite de ce phénomène, il se produit une adhérence intime entre les macrophages de l'exsudat et les hématies qui présentent un aspect bizarre. En examinant avec un faible grossissement, on trouve dans la goutte de l'exsudat des grumeaux qu'on serait tenté de prendre d'abord pour une vraie agglutination. Mais l'observation plus précise démontre aussitôt la fausseté de cette interprétation, et nous apprend qu'il s'agit ici d'amas de leucocytes mononucléaires, qui sont accolés à une quantité d'hématies. Dans cet état, les deux espèces de globules peuvent persister/

* Resorption des cellules. Annales de l'institut Pasteur 1899. pages 743 & 744.

persister assez longtemps, et ce n'est que les macrophages finissent par englober les hématies entières dans leur protoplasma."

(28)

Savtchenko has made the same observation as Levaditi.

(26)

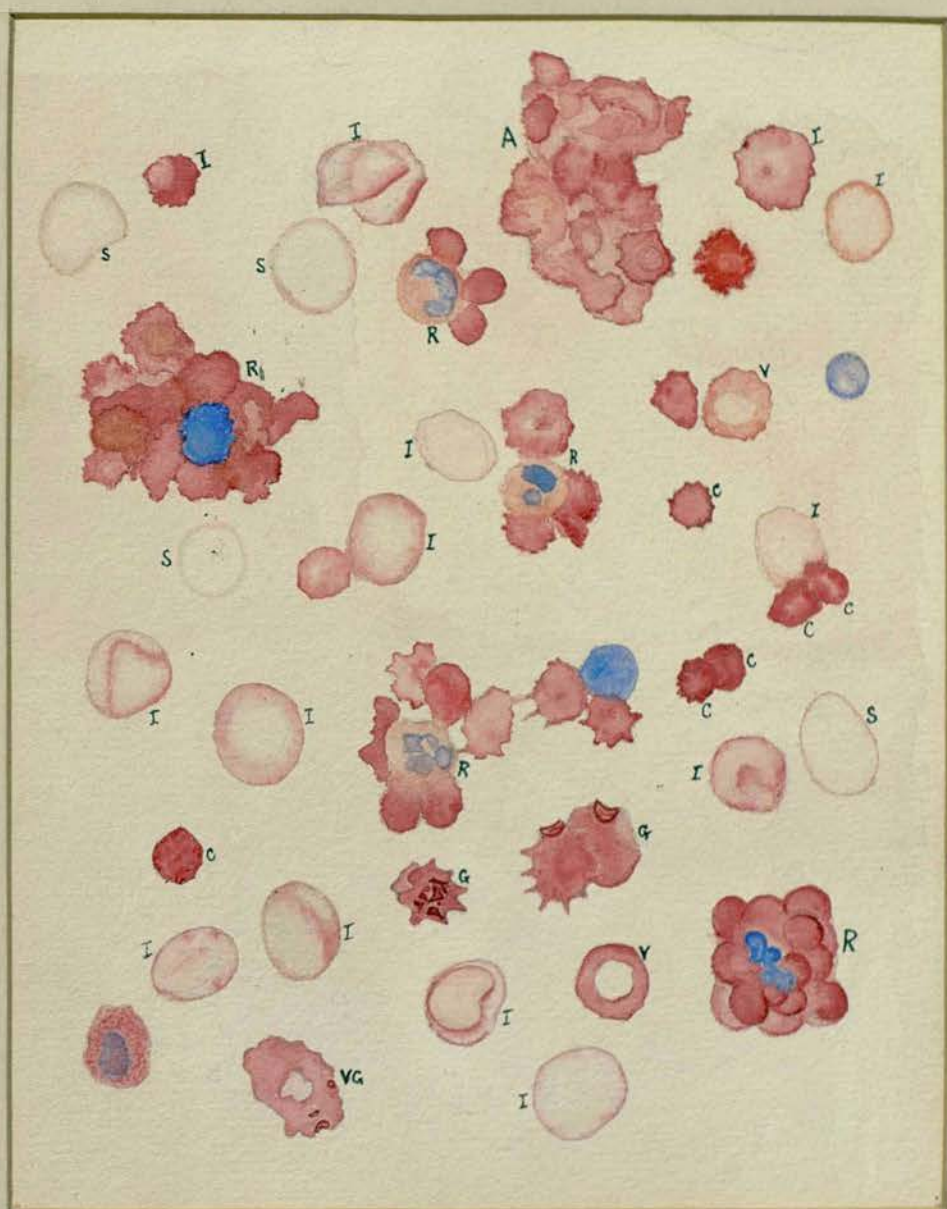
Gruber conducted many experiments to test this and other observations made by Levaditi. He entirely agrees with Levaditi in maintaining "that erythrocytes, which contain Intermediary Body, undergo Phagocytosis much more quickly and in much greater number than do normal erythrocytes."

Gruber also found that leucocytes which have been previously treated with a haemolytic serum act specially energetically. These experiments of Gruber will be described at greater length hereafter when the fate of the corpuscles and haemoglobin thus absorbed is being considered.

Ruziczka has also noted this extraordinary Phagocytic attack on the red corpuscles in the presence of Intermediary Body.

(29)

Ruziczka immunised guinea pigs to fowls blood and then after immunization, the fate of the blood corpuscles of later intra-peritoneal injections was ascertained/



- R. = Ruziczka phenomenon.
 Rl. = the same but atypical.
 A. = Agglutination.
 S. = Shadow Cells.
 I. = Cells showing various degrees
 of Haemolysis.
 V. = Vacuolated Cells.
 G. = Cells showing globules of
 dissolved Haemoglobin.
 C. = Small cells showing no evidence
 of Haemolysis.

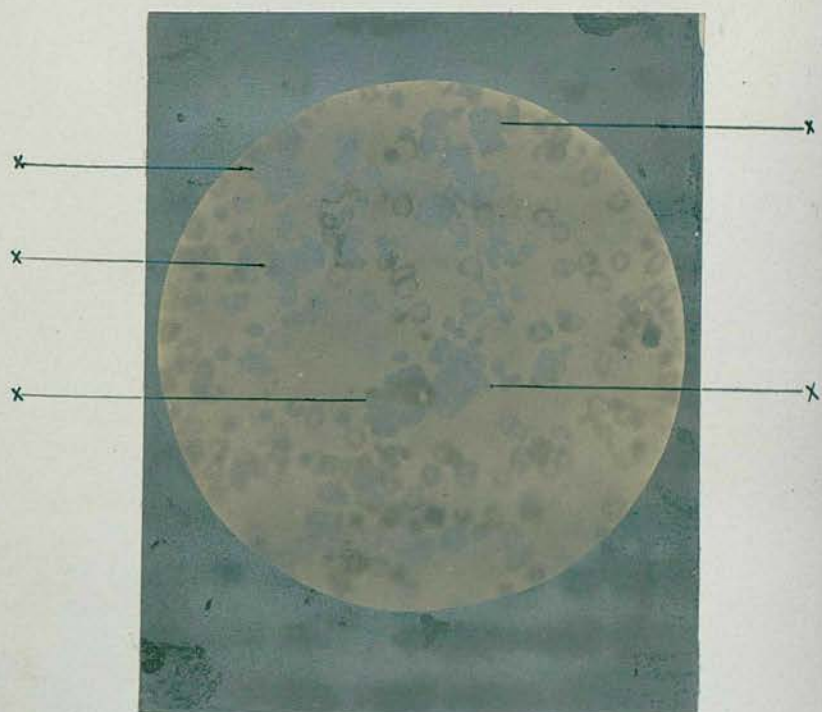
ascertained (after some time had elapsed) on obtaining some peritoneal contents by means of a capillary tube. He observed that the microphages and macrophages are able to destroy the red corpuscles without previously absorbing them, but by fixing themselves to the red cells and gnawing and digesting them away bit by bit, apparently by the action of a digesting juice.

Those corpuscles which are thus nibbled away do not swell up (as when destroyed free in the serum by the complement) nor do they lose their haemoglobin, but retain it to the last and indeed possess a more intense colour than the intact corpuscles. This process can be followed beautifully in vitro.

The above description is a literal translation from Gruber who has completely satisfied himself of the accuracy of Ruziczka's observation.*

I have said that in the microscopical examination of normal human blood which had been exposed to the action of paroxysmal haemoglobinuric serum, I had made an observation which concerned jointly the leucocytes and erythrocytes. It was this phenomenon described by Ruziczka and Gruber to which I referred, and the reason I deemed my observation of importance was that which I have just stated viz. that Levaditi, Savtchenko, Gruber and/

* Ruziczka's work is published in the Bohemian Language.



and Ruziczka are agreed that such unusual eagerness of the Phagocytes is the result of the activities of Intermediary body on the red corpuscles.

Figure I. (page 38.) and my camera lucida drawing (page 41) indicate the similarity of the process, as I have observed it in Paroxysmal Haemoglobinuria, to that observed in animals by Gruber and Ruziczka.

I also submit a photograph (opposite page) and microscopical preparation (slide 3) which likewise show this extraordinary leucocytic activity. The apparent indifference of these latter preparations is due to the destructive changes that have occurred in consequence of the haemolytic process. The illustrations and specimens all show the deeper colour possessed by those red cells which are attached to the leucocytes. It may also be seen that those attached cells have not become enlarged.*

In many cases also the red cells appear to be partly nibbled away. The significance which I attach to those changes which I have observed in the red and white cells is that we have probably to do with the activities of ^{an} intermediary body in the serum of individuals affected with Paroxysmal Haemoglobinuria.

November 18th. T.S. was blistered on the previous/

* Metchnikoff gives a diagram to illustrate his views which I have already quoted. (loc.cit.page 764).

previous evening. Twenty-four hours before the application of the blister a paroxysm had occurred. A portion of the serum was kept at a temperature of 56° Cent. for half an hour to inactivate the thermolabile substance (complement).

Now for the first time I used a 5% suspension of washed corpuscles. This was made from normal blood (5%) in sterile .5% Na Cl solution, after shaking up thoroughly, centrifuging, drawing off the salt solution with a pipette, and replacing salt solution to the original bulk. The process was repeated three times. My blood was used.

1 c.c. active serum 1 c.c. 5% mixture washed corpuscles.

11 c.c. inactive serum 1 c.c. 5% mixture washed corpuscles.

These were kept at 37° cent. for 3 hours without naked eye evidence of haemolysis in either case.

The result was likewise negative after 24 hours the remaining 21 hours being at room temperature.

As several new factors were introduced on this occasion the negative result could not be definitely attributed to any one.

It did not appear to me that washing of normal corpuscles could have anything to do with the negative/

negative result. The important change in the conditions was the temperature.

At the blood temperature the haemolysis of animals corpuscles by an immune serum is much more rapid than at the temperature of the atmosphere. I was therefore not unprepared to find that haemolysis would occur in the above experiments, within a shorter period than had hitherto been the case at the room temperature.

In this experiment however the haemolysis was so much retarded that none was obvious to the naked eye at the end of 24 hours. This result indicated the probability that the temperature conditions of my earlier experiments were more suited to the activities of the haemolytic substance, and I also recognised that they more nearly resembled the clinical conditions of a paroxysm.

November 19th. The same serum now a day old was used in a similar way. A 5% suspension of washed blood corpuscles was made from the blood of M.R., (another case of Paroxysmal Haemoglobinuria.) The latter had two severe paroxysms on the 17th and 18th. The following tests were made

(1)/

- (1) 1 c.c. active serum .75 c.c. 5% suspension
 *
 W.B.C. in .5% salt solution

No haemolysis in 5 hours at 37°C.

- (2) 1 c.c. inactive serum .75 c.c. 5% suspension
 W.B.C. in 5% salt solution

Result ditto.

- (3) 1 c.c. .5% salt solution .75 c.c. 5% ditto.

(Control)

Result ditto.

Again the conditions of temperature appear to have
 been unsuitable to the production of haemolysis.

November 24th. I obtained blister serum from Mrs. W. a case of Pleurodynia and blood from T.S. and M.R. cases of Paroxysmal Haemoglobinuria. From M.R. the blood was obtained five hours after she had passed porter coloured urine and from T.S. when he was beginning to feel cold and miserable. One hour thereafter he passed porter coloured urine. Blood was therefore obtained during the paroxysm. It was obtained with considerable difficulty, his hands being cold, blanched and cyanotic at parts. The following preparations were then made.

* W.B.C. = Washed blood corpuscles.

			Time 5 hours		7 hours
37°C.	(1)	Serum (.5 c.c.) + T.S.'s blood (2 drops)	No haemolysis		No H
	(2)	" " + M.R.'s " " " " "			trace
	(3)	" " + T.S.'s " (.5 c.c. of 5% corpuscles in 5% NaCl)	" "		No H
	(4)	" " + M.R. " " " " "			trace
	(5)	" " + self " " " " "			No H
	(6)	5 c.c. of 5% NaCl solution + T.S. " " " " "			trace
	(7)	" " + M.R. " " " " "			trace
	(8)	" " + self " " " " "			No H.

The serum had therefore no haemolytic action on my corpuscles. Any haemolysis which occurred in the blood of T.S. and M.R. was no doubt due to changes which had been already initiated in vivo during the paroxysm.

December 5th. Blister serum was obtained from T.S. during a paroxysm. On spectroscopic examination the 3 bands of methaemoglobin were observed. The colour of the serum was olive brown. The following combinations were placed in the incubator for 5 hours.

(1)	<u>Active</u>	serum + normal washed corpuscles 5% in 5% NaCl sol.	No Haemolysis
(2)	"	" + " fresh blood	" "
(3)	<u>Inactive</u>	" + " " "	" "
(4)	<u>Active</u>	" + " " 5% in 5% NaCl solution.	" "

The temperature of the incubator as I had already found, retarded the haemolytic process.

December 24th. Blister serum of T.S. (no paroxysm) + corpuscles (as above) 7 hours in incubator.

Negative result.

December 18th. Blister serum from a case of myxoedema.
1 c.c. serum + normal blood (3 drops) no haemolysis
in four days at room temperature. Microscopic examination shewed cells unchanged.

Blister serum from a case of Rheumatoid Arthritis.
1 c.c. serum + normal blood (3 drops) No haemolysis
until 4th day.

BLOOD SERUM EXPERIMENTS.

A few blood serum experiments were done but these were not quite satisfactory as the serum obtained was usually much coloured with Haemoglobin.

November 3rd. Case T.S. The skin was prepared by washing and a boracic poultice was applied over night. The skin was again washed with sterilized salt solution before withdrawing blood, which was obtained by wetcupping. The cupping machine was washed in carbolic lotion and then with sterilized salt solution. The cupping glasses were boiled in salt solution for ten minutes. The blood in one of/

of the glasses was allowed to stand till the serum separated. The glass was meanwhile covered with corrosive wool. The serum was somewhat deeply coloured. The blood in a second glass was used to make a 5% suspension of washed blood corpuscles in .5% NaCl solution. A similar preparation was made of my own corpuscles.

- | | |
|--|-------------------------|
| (1).75 c.c. serum + 1 c.c. 5% W.B.C. of T.S. | } 1 hour
at
37°C. |
| (2).75 c.c. " + 1 c.c. " " " " of myself | |
| (3).75 c.c. control + 1 c.c. " " " " of T.S. | |

No naked eye evidence of haemolysis was obtained in this period but the original colour of the serum obscured the result. Microscopic preparations, made after the tubes had stood for 11 hours more at the room temperature, indicated that the cells in (3) were much more numerous than in (1).

Ruziczka's phenomenon and also great variation in the size and colour of the cells were noted in Nos. 1 and 2. The cells were now (after 11 hours) much less numerous than when first examined (after 1 hour).

November 9th. Blood serum was obtained in the same way from T.S. A 5% suspension of his washed blood corpuscles in .5% NaCl solution was then made. For the/

the latter purpose blood was obtained from the ear. A drop of the corpuscle and salt solution mixture was placed on a glass slide and a cover slip was applied. The cells were all decidedly crenated.

A drop of the serum was then allowed to mix under the margin of the cover glass. The cells which came in contact with the serum were observed first of all to lose their crenation, later they became swollen while losing their crenation and finally they became irregular in shape.

This irregularity quickly increased until many had apparently joined together forming shapeless masses or a network. This process was completed in half an hour at the room temperature and I watched the process from start to finish. By this time there were also many colourless shadow cells and the leucocytes could be seen surrounded by red cells (Ruziczka phenomenon). I repeated this experiment several times and always with the same result. Normal blood (my own) was then prepared in the same way and a drop of T.S.'s serum allowed to pass under the edge of the cover glass. Exactly the same process occurred but it took a definitely longer time, about 10 minutes longer.

1 c.c./

1 c.c. serum + 1 c.c. 5% normal W.B.C. in 5% saline solution kept 3 hours at blood temperature gave no naked eye evidence of Haemolysis in the tube.

The observation made by the microscope indicated that destruction of the cells was proceeding very rapidly while the tube preparations after a much larger period showed no evidence of haemolysis. It will be noted that the temperature conditions were not the same in both cases.

These and my previous observations indicate that a potentially toxic substance is present probably at all times in the serum of individuals affected with Paroxysmal Haemoglobinuria. The destructive action of this substance on blood corpuscles is not apparent at the normal body temperature.

Exposure for some time to a low temperature appears to be a necessary condition for haemolysis by this substance.

Permanent preparations of the blood, from which this serum was obtained, shewed the Ruziczka phenomenon very well.

November 12th. Blood serum obtained from the patient M.R. during interval. Under aseptic precautions blood was obtained from a vein of the arm, by means of an exploring syringe. The blood was/

was allowed to coagulate in the syringe and when the serum had separated it was drawn up by means of a fine pipette. The serum was clear and yellow.

The serum was diluted with an equal part of 5% NaCl solution in order to have a workable quantity of fluid. One half of this was inactivated by keeping it at 56°C. for an hour.

- | | |
|---|---|
| (1) .75 c.c. active serum + .75 c.c. 5% mixture of normal W.B.C. in 5% NaCl solution | } <u>distinct</u>
<u>Haemolysis</u>
<u>in 3 hours</u> |
| (2) .75 c.c. inactivated " + .75 c.c. 5% mixture of normal W.B.C. in 5% NaCl solution | |
| | } <u>No</u>
<u>Haemolysis.</u> |

The tubes were exposed to the room temperature for $\frac{3}{4}$ hour before being placed in the incubator.

Hitherto I had rarely obtained haemolysis as quickly and, in the course of three hours, never in such a pronounced degree. The negative result obtained in tube 2 was a corroboration of my supposition that the haemolysis was effected by means of Intermediary body and complement.

I have already indicated my reason for believing that an Intermediary Body was present in the serum. (page 42) This is a thermostabile substance as Ehrlich and many subsequent writers have demonstrated. It remains uninjured by exposure for $\frac{1}{2}$ hour to a temperature of 56° C.

I expected however to be able to prevent
haemolysis by rendering inactive a second substance
viz. the complement or thermo labile component
of the haemolysin. The result as shewn by 2 agreed
with my anticipation.

Microscopic examination shewed the cells in (1)
 in all stages of destruction. Free haemoglobin
globules in the cells were to be seen and the
Ruziczka phenomenon present.

In tube (2) the cells were much more normal and
apparently contained more haemoglobin. Although a
 slightly hypotonic salt solution was used in the
 above experiments, the positive result in (1) is
 not invalidated thereby for the reason that
 haemolysis of normal corpuscles, so prepared, has not
 occurred within three hours in my experience, nor
was there haemolysis in (2).

I had the intention to reactivate the inactivated
 serum of M.R. by adding fresh normal complement,
 but there was not sufficient of M.R.'s serum
 available. I regret this as my experiments were
 interrupted shortly after this, by consideration of
 the patient's feelings and condition.

December 1st. Under aseptic precautions
 blood was obtained from an arm vein of T.S. (interval)
 by/

by means of an exploring syringe. Serum was obtained by allowing the blood to stand at the room temperature until separation from the clot had occurred. It was well coloured.^{*} Too little was obtained to make experiments in tubes.

Films of my blood were made and when these became dry, the serum of T.S. was here and there applied in small spots to the surface of the film. These were allowed to dry. The films were then stained and mounted. Permanent specimens were thus obtained shewing the effect of the serum at room temperature, on the cells of the dry film. Evidence of the dissolving action of the serum for normal corpuscles was thus obtained.

This ended the series of experiments performed during 1903 and on consideration of the results which were obtained I was enabled to arrive at the following conclusions.

- (1) A pathological substance is present in the blood serum and lymph of individuals affected with Paroxysmal Haemoglobinuria.
- (2) This substance can dissolve (in vitro) the corpuscles of the affected individual (autolysis) and also /

* Carmine

and also those of normal individuals (iscolysis) provided suitable conditions as to temperature exist.

- (3) Temperature conditions require to be further studied; it is however apparent that a temperature, much below that of the body, favours the action of the haemolytic substance while the body temperature retards or prevents it.
- (4) My observations regarding Phagocyte activity during the Haemolysis (in vitro) correspond with those made by Levaditi, Savtchenko, Gruber and Ruziczka, in their comparative work on Immunity.
- (5) These authors consider that the excessive Phagocytic action, observed by them, is significant of the antecedent union of Intermediary body with the red corpuscles.

 "The Ruziczka phenomenon" is constantly observed during the autolysis ^{and} iscolysis of red corpuscles produced by the serum of Paroxysmal Haemoglobinuria cases. Therefore the probable explanation of the Haemolysis is the union of Intermediary Body and Red Corpuscles.
- (6) The Intermediary Body of Paroxysmal Haemoglobinuria however cannot of itself produce solution of red corpuscles /

corpuscles. For this, the presence of a thermo-labile substance complement, is also required (Pages 50 & 51).

- (7) The changes observed in the red cells in course of solution correspond with those produced by the Haemolysis of an Immune serum as described by Gruber.
- (8) The serum of normal individuals does not cause haemolysis. The serum of various individuals suffering from disease produced little and generally no haemolysis.

The following scheme indicates my interpretation of the foregoing experiments.

	normal corpuscles	Pathological Serum.	Temperature at 10-15°C.
I.	R.B.C.	+ (I.B.+ C)	
forms the following combination			
II	(R.B.C.+I.B) (sediment)	+ C (fluid serum)	
III.	R. B. C.	+ do. (decanted from II)	at 37°C. <u>No haemolysis.</u>
IV.	R. B. C.	+ do. (decanted from II) + I.B. (inactivated serum of T.S.)	at 37°C. <u>No haemolysis.</u>
V.	No. II.		at 37°C.
forms the following combination			
	(R.B.C. + I.B. + C)		<u>Haemolysis.</u>

As my experiments for the present were interrupted the above scheme, which I have found in my notes of this date, remained without further experimental proof than that which I have already submitted.

The views embodied in the scheme are entirely supported by facts already ascertained regarding the mode of action of Intermediary Bodies and complement. (30)

On examining the literature I found that three months before the beginning of the present research (31) Professor R. Kretz, had published a "Vörläufige Mittheilung " on the results of an investigation, which had the identical object of my own work viz. to discover if the presence of a haemolysin in the blood of Haemoglobinuria cases accounts for the solution of the erythrocytes.

With this object in view he obtained blood from a case of Paroxysmal Haemoglobinuria and also from cases of gastric ulcer, nephritis and herpes zoster. The blood was obtained from an arm vein in all cases. Before taking blood from the herpes zoster case the arm, from which the blood was to be withdrawn, was chilled by plunging it in cold water. He prepared a 5% suspension of corpuscles in salt solution. The strength of the salt solution employed and the method by which the serum was separated from the corpuscles are not stated. He found that the corpuscles of the Paroxysmal Haemoglobinuric case were completely or almost completely dissolved by the serum from the same individual/

individual. A similar result however was obtained by using even smaller proportions of serum from the other cases especially the case in which the serum was obtained after previous chilling of the limb.

The corpuscles of the control cases, on the other hand, were dissolved to a less extent (1) by Paroxysmal Haemoglobinuria serum (2) by the various control sera (3) by the inactivated serum of the Haemoglobinuria case + fresh serum (4) by the serum from the Herpes Zoster case(chilled limb) which had in this instance no stronger action than the other control sera.

He concluded that (1) the corpuscles of the Haemoglobinuria case were loaded with Intermediary Body and contained more than the corpuscles of all other tested cases. (2) The serum in Paroxysmal Haemoglobinuria contained no free Intermediary Body. (3) In the fresh serum from the blood of the chilled arm (Herpes Zoster case) there is a distinct increase in the amount of complement in comparison with serum obtained without previous chilling. (4) Further the haemoglobinuria paroxysm is the effect of an increase of complement through the thermic influence upon the blood in the blood vessels of /

of living individuals "with riches of amboceptors" (Intermediary Body).

Kretz does not indicate how long the tubes were allowed to stand, before reading the results, positive or negative. The blood was obtained from the Paroxysmal Haemoglobinuria case in the "attack-free" period.

These conclusions are in a very essential way opposed to mine. There is agreement to the extent firstly, that the toxic substance acts by means of an Intermediary Body and complement and secondly, that cold produces some change which permits the destructive process to occur.

I am absolutely opposed to Kretz in his contention that the red cells, during the "attack-free" period, are loaded with Intermediary Body and that the paroxysmal destruction of the red cells results from the increase of the complement to the serum caused by the action of cold.

I maintain that Intermediary body does not become anchored to the cells except as the first step of an immediately ensuing haemolysis, and that the anchoring of free Intermediary Body is determined by the action of cold.

Kretz as well as Luzatti and Sorgente have committed/

committed themselves to the opinion that the change, which they assume to occur in the plasma from the action of cold, can only occur in vivo, but this view is probably wrong, even by the latter authors' own showing. They obtained a coloured serum by standing blood obtained from their patient. firstly on ice and then in the incubator. To this observation they attached no importance because they obtained similar results with normal blood.

(32)

Ernest Burckhardt agreeing with Luzzatti and Sorgente found no abnormal haemolytic activity in the interval-serum. The corpuscles had no diminished resisting power to cold in vitro. If however, one of the extremities was immersed in cold water before abstracting blood, then the serum separated with a strong red colour. This colouration of the serum was also observed, though in a less degree, if the limb were merely ligatured without being placed in cold water. If the limb while ligatured was at the same time kept warm the serum obtained was not red in colour. He agrees with Luzzatti and Sorgente firstly in considering that stasis is only important in so far as the limb consequently/

consequently becomes cold, and secondly in considering that subtle changes in the plasma then come into play as is also indicated by the change in the mode of coagulation.

Finally he alludes to the possibility of a ferment as being the cause of the blood changes.

Camus in his Thesis did not occupy himself with an investigation of the properties of the serum, but he thought it permissible to suppose that autolysines or auto-sensibilisatrices capable of producing a great destruction of red cells and causing haemoglobinuria may be produced in the human body. (Loc.cit. page 15)

(33)

Mattirolo and Tedeschi made an elaborate investigation of two cases, one of which was a typical Haemoglobinuria a frigore (A) and the other was a case in which muscular effort induced paroxysms and there was a history of syphilis (B)

They tested the agglutinating and lytic powers of the serum. Aseptic precautions were observed as much as possible. The serum was obtained by allowing the blood to coagulate and the serum to separate from the clot at room temperature.

It must be said that by such means the toxic properties of the serum cannot be ^{fully} retained.* It was/

* This statement is proved by experiments which I shall describe later.

was at this temperature that I obtained haemolysis and I assumed the haemolysis was due to the absorption of amboceptors from the serum by the corpuscles. The serum must therefore have lost its distinctive characteristic, in a great degree, by standing so long in contact with corpuscles at the room temperature. To 5 c.c. of a 5% suspension of washed blood corpuscles (of various individuals) in .85% NaCl solution they added varying quantities of serum. The tubes were put in the incubator for an hour and then were kept for 23 hours at the room temperature. The serum was not always fresh as "it was never used later than 2 days after abstracting the blood." The serum and corpuscles of a case of chronic nephritis and one of spasm of the diaphragm were available for control experiments and for testing isolytic as well as autolytic properties of the various sera.

The control sera had no auto- or iso- haemolytic action. The interval serum of A was autolytic and was also isolytic for normal corpuscles and those of the nephritis case but not for B's corpuscles. The attack serum (the serum was to begin with haemoglobinaemic) had no autolytic nor isolytic property. The interval serum and also the paroxysm serum/

serum of B had no haemolytic action. The corpuscles of the control cases ^{were} dissolved by A's interval serum, but not by B's serum, nor by nephritic serum.

The interval-corpuscles of A were not dissolved by normal or nephritic ^{serum}, nor by B's or A's attack-serum but by A's interval-serum. A's attack corpuscles resisted all sera. B's corpuscles at all times resisted solution. In the positive results above indicated the serum sometimes showed rose colouring, but never became ruby red. The active properties of the serum vanished if heated to 55° C. for $\frac{3}{4}$ - 1 hour.

Mattirolo and Tedeschi consider that auto- and isolysis can therefore be demonstrated in vitro.

They inclined to believe that there was an antitoxic substance formed during the attack which, by becoming joined to the free toxic substance, accounted for the diminished destroying power of the serum or by attaching itself to the cells, so rendered them less susceptible to the toxine. They further consider that in this way cell destruction is prevented between the attacks. They supported this contention because of their observation that the cells in the intervals are more resisting to agglutination by an active serum than are normal cells/

Include

cells. This argument is of doubtful value in view (1) of the unascertained relation of agglutination to lysis, (2) of the authors' own experiments (page 378), which show that the corpuscles of the intervals are no more resisting to lysis than normal corpuscles. One can readily agree with them that the equilibrium must be maintained by two opposing forces viz. the toxicity of the serum and the resisting power of the corpuscles. It is, however, an unwarranted speculation to hold, as they do, that the degree of toxicity of the serum, or the resisting power of the cells, depends on the formation of an antihaemotoxin which could prevent paroxysms or limit their duration. The authors were unable to show that the corpuscles were less resisting to agglutination (the possible fallacy here has already been indicated,) or that the serum was more toxic even so early in the paroxysm as 10-15 minutes after the first rigor. The explanation of those latter (negative) results which they suggest is that the antihaematoxin forms so quickly as to prevent an opportunity for proving their theory.

According to Mattiolo and Tedeschi the pathogenesis of case B is entirely different as (1) the serum had no haemolytic action (2) it never became /

became haemoglobinaemic either between or during paroxysms and lastly (3) paroxysms were produced by exertion and not by cold. In this class they believe "there is a pathological congestive condition of the kidney, analogous to what some recognise in order to explain orthostatic albuminuria." They also assume an increase of the toxicity of the serum in consequence of this condition of the kidney and of the increased muscular exertion.

(34)

Donath found that the serum of the interval and also of the attack had comparatively little auto- or iso-haemolytic action, in three cases investigated. The serum of one case had moreover no haemolytic action on another case. He maintained that such iso-haemolysis as he observed with interval-serum could in no way explain the haemolysis of attacks unless it be proved that the attack-serum has a much greater power. He considered that these practically negative results, in vitro, did not negative the presence of Haemolysin, since its opportunity for becoming anchored to the cells in vivo must always be so favourable as to make its presence no longer demonstrable in vitro, unless when/

when present in great excess. He could not prove any excess of amboceptor or of complement during the paroxysm. As the result of some careful experiments he concluded that if a haemolysin component is present in the serum it is probably a pathological substance peculiar to the blood of this disease.

He found that the corpuscles both of the paroxysm and of the interval behaved exactly like normal cells. This result corroborates the experiments I have already done and contradicts the conclusions of Kretz.

Stasis serum from a case of chlorosis had no haemolytic action either on Haemoglobinuria blood corpuscles nor on those of two control cases.

Donath repeated Kretz's experiment of cooling a limb before withdrawing blood from which to obtain serum.

Sometimes but not always ^{haemolytic} the power of the serum was slightly increased by this proceeding.

Experiments have been done by several workers, with the object of studying the effect of cold on the blood and urine in animals. Long ago, Lichtheim⁽³⁵⁾ failed to produce haemoglobinuria in rabbits by putting a large part of the animal in a freezing mixture (page 1163).⁽³⁶⁾ Reineboth and Kohlhardt subjected rabbits to cold and found that the Haemoglobin diminished much more than the corpuscles. Haemoglobinaemia but not haemoglobinuria was produced.

⁽³⁷⁾ Luzzatti and Sorgente by putting rabbits in a cold bath at 3 - 4° C. for 15 minutes found that in some cases the rabbits died. The internal organs were greatly congested and the great internal vessels were much dilated. The blood was very dark and thick. The serum, when separated from this,

showed no bands of Haemoglobin on spectroscopic examination. They did not find much diminution in the Haemoglobin of the blood and the corpuscles were not diminished but increased, as they generally are in stasis blood. Coagulation^{of the blood} and separation of the serum occurred "immediately" while in control rabbits this process took an hour.

The corpuscles of the chilled rabbits tended to agglutinate in normal serum, while normal rabbits corpuscles did not agglutinate in the serum from the/

the chilled rabbits.

The corpuscles of the chilled rabbit in its own serum showed some tendency to colour the serum.

L. & S. inclined to believe that, in healthy animals, a change in the coagulation process and perhaps some changes in the serum or plasma occurs as the result of the action of cold.

Camus reduced the temperature of a dog for 5 hours, during two of which the rectal temperature averaged 28° C. About the midterm of these two hours there were "perhaps traces of haemoglobin in the plasma." Later there was albumin in the urine but no haemoglobin. (Loc.cit.page 19)

The influence of cold in relation to Paroxysmal Haemoglobinuria is so important that no theory of the pathology can be considered satisfactory unless this relation can thus be explained. Naturally much research has been concerned with this aspect of the subject and ingenious suppositions have been plentiful in consequence of the perplexingly varied results of experiments. Fortunately the technique has so much improved as to expect more uniform results in future. /

in future.

Cold in relation to this disease has been investigated

(a) in vitro

(b) in vivo

(a) In vitro experiments have been conducted

(1) on the blood

(2) on the corpuscles

(3) on the serum.

(1) In the experiments on blood sometimes a destructive effect on the corpuscles was indicated by the colour which the serum acquired but in the majority of cases the results were negative, so far as the colour of the serum indicated.

As will be seen later these destructive changes in the corpuscles are not necessarily the result of a pathological diminution of their resisting power to cold. The action of the serum is not excluded by such an experiment. (2) To determine the resisting power of the corpuscles to cold, the serum must be removed as has been done in the careful work of
Ehrlich⁽¹⁹⁾ and of Donath,⁽²⁵⁾ and Donath and Landsteiner.⁽³⁸⁾
The exact methods of these authors renders their conclusions of great importance.

* In the experiment by Ehrlich the serum was considerably diluted by saline solution.

According to them the corpuscles of Paroxysmal
Haemoglobinuria cases resist the action of cold

like normal corpuscles. I shall return immediately to a fuller consideration of in vitro experiments.

(b) In vivo experiments have been conducted by
immersion

(1) of the body

(2) or a portion of the body
in cold water.

(1) In the former there is of course uniformity in the results so far as typical cases of Haemoglobinuria a frigore are concerned. I shall deal with the class of cases in which paroxysms are precipitated by muscular exertion in another place. (2) In experiments conducted on a limb, finger, etc. (with the exception just indicated) results again uniformly showed, that the serum became haemoglobinaemic. As in the case of the
in vitro experiments on whole blood one cannot
conclude that the haemoglobinaemia and haemoglobin-
uria were the direct result of the action of cold
on the corpuscles. There is the third factor - the serum- which is not eliminated in such experiments.

The action of cold on the serum will presently be /

be referred to.

Having regard to the fact, firstly, that the washed blood corpuscles of Paroxysmal Haemoglobinuria have a normal resistance to the action of cold*, and having regard to this further fact that those corpuscles do not resist but become dissolved when similarly exposed in the form of whole blood, it becomes evident that the presence of the serum is necessary in order that cold may produce its injurious effect.

How then does cold produce this effect?

The work of Moreschi⁽³⁹⁾ has demonstrated that the isolytic action of human serum depends on two substances, one of which is bound to the corpuscles in the cold and the other remains free in the serum. The latter (complement) is also present in the serum of normal human blood, which is not isolytic. Isolytic human serum becomes ineffective by heating to 40 - 48° C, in consequence of the destruction** of complement at this temperature. Nevertheless the haemolytic action is restored by the addition of fresh non-isolytic human serum.

Moreschi's observations regarding the method of action of human isolytic serum support my own conclusions regarding the particular case of P.H.

Finally/

* page 69

** or inactivation.

Finally my conclusions have been absolutely confirmed and supplemented by Donath and Landsteiner ⁽³⁸⁾ whose experiments, published in September 1904, were carried through with the most perfect technique.

They obtained blood from one or other of their 3 cases and mixed it with an equal quantity of a .25% solution of Potassium Oxalate in .85% NaCl solution, in order to prevent coagulation. Aseptic precautions being observed,

- (a) a portion was kept in the incubator for 3 hours and the serum was then clear yellow :
- (b) a second portion was kept standing in ice-water for half an hour and then placed in the incubator for two hours. The serum was then ruby red:
- (c) a control of washed blood corpuscles from the patient was treated like (b) and gave a negative result:
- (d) serum + corpuscles with similar temperature changes gave a ruby red colour of the serum, but, if the ice-water stage was omitted, the serum remained clear yellow.

Maintaining the serum for $\frac{1}{4}$ hour at 45° C. checked its action almost completely, and 55° C. completely/

completely. Thus there is a thermolabile
constituent of the toxine, the so-called complement,
as I have already proved ~~(Paged 50 and 51).~~

They further showed that the serum during the
period of cold lost something to the corpuscles,
so that it had no longer any haemolytic action on
the patients own corpuscles nor on those of other
individuals. If the mixture was not cooled
before the separation of the serum had taken place the
latter
still retained its haemolytic action on fresh
corpuscles.

Again the active properties of the serum are
 shown also in mixtures with the corpuscles of other
 individuals, provided the mixture is previously
 cooled on ice for half an hour. If not so cooled
 there is little or no haemolysis in 3 hours at 37° C.
If, immediately after standing in ice, the serum is
removed by centrifuging and the original bulk is
restored with 1% NaCl solution, no haemolysis takes
place. If, instead of salt solution, an unchanged
active serum of another individual is added,
haemolysis takes place. Cooling of corpuscle-free
plasma does not confer on it the property to act
as a haemolytic nor are serum-free corpuscles more
easily dissolved after being cooled.

Dilution/

Dilution of the serum 4 - 8 times with salt solution in one case deprived it of haemolytic power. The corpuscles of Haemoglobinuric cases + serum of other individuals first of all cooled on ice and then kept for two hours at blood temperature gave negative results.

The serum of Haemoglobinuria patients therefore contains a lytic substance (for human corpuscles) which cannot be demonstrated except under suitable conditions of temperature, as, at the body temperature, it does not combine with the blood cells or only to a slight degree. The union formed in the cold between Intermediary Body and cells does not yield (or only slowly) when the warming process takes place in vivo or in vitro, so that meanwhile the so-called complement enters into the combination and hindering the dissociation haemolysis thus results.

They investigated also the degree to which cooling must take place in order to obtain positive results. In one case, a temperature of 5°C for ½ hour and in another 10°C. for ½ hour sufficed. If the mixture was kept at the room temperature for this period only traces of Haemolysis occurred.

Donath and Landsteiner received the verbal assurance of Kretz that in his experiments there was inadvertently a period of cooling during his experiments.

Having familiarised myself with the excellent technique of Donath and Landsteiner, I determined to begin experiments not only with the object of confirming the work which they had done, but in order also to confirm my own especially regarding the properties of the blister serum. My object in doing so was because I hoped to proceed later with an attempt to produce an antitoxin or anti-intermediary body. I foresaw that the amount of serum, required for repeated injections into animals, would be obtained most easily and with least inconvenience to the patients by employing blisters.

Experiments with blood serum of the Interval.

October 1st. 1904.

Blood was obtained from the patient T.S. by puncturing a finger. No bandage or tourniquet was employed. The blood was mixed with two parts of a .25% solution of Pot. Oxalate solution in .85% NaCl solution

- (A) Twenty drops of this mixture were put in a small sterilized test tube which was then placed in ice water for half an hour and thereafter in an incubator (37° C.)

(B)/

(B) A similar preparation was placed in the incubator. Ice water stage was omitted. No haemolysis occurred in either in 9 hours. At end of 24 hours when the next observation was made haemolysis had occurred in A but not in B.

Landsteiner and Donath obtained haemolysis in a preparation corresponding to A in $2\frac{1}{2}$ hours. There are two ways by which to explain the relatively slow haemolysis occurring in my experiment.

(1) The haemolytic substance of the serum may have already been of low concentration in vivo.

(2) It was artificially more diluted than in the experiments of Donath and Landsteiner.

Two parts of oxalate solution to one of blood were used by me instead of equal parts.

The positive result in A and the negative result in B taken together show that cold was a determining factor in the production of haemolysis.

(C) Simultaneously a control experiment with the washed blood corpuscles of the patient was done.

A tube containing the original blood-oxalate mixture was placed for half an hour in ice water, the mixture was then centrifuged and the fluid removed, .85% ^{salt} solution was added to the sediment in the tube, lightly shaken and centrifuged, fluid again removed. This process was repeated three times and/

and finally salt solution was added to bring to the original bulk. The tube was then placed in the incubator. No haemolysis after 24 hours. The corpuscles now resisted solution by the action of cold.

Therefore in order that haemolysis may result the serum must be in contact with the corpuscles not only during the cold period but also during the following warm period. Such a conclusion is in agreement with what is already ascertained regarding the inertness of complement at the temperature 0° C. (2&30) If therefore the serum be removed at the termination of the ice water stage, haemolysis will not ensue during the later warm stage, although the red cells and Intermediary Bodies have become locked together.

October 4th.

Blood-oxalate mixture prepared as before from blood of patient between attacks. A portion was placed in ice water for half an hour, centrifuged, the serum (a) drawn off into a sterilé glass tube and the corpuscles (b) made up to original bulk with salt solution. A second portion of blood oxalate mixture, kept warm, was centrifuged, serum (c) drawn off and the corpuscles washed in warm salt solution and centrifuged three times and finally made up to original/

original bulk with .85% salt solution. Blood
 (d)
 corpuscles were thus obtained which had been
 immediately washed from the serum at the blood
temperature (as nearly as possible.)

I. a + d were placed in a tube in ice water for half
 an hour and then in incubator for 24 hours. No
haemolysis. When this experiment is compared
 with Experiment A (October 1st) one considers that
 the serum a has lost its power to dissolve cor-
puscles d probably by giving something up to the
corpuscles^b with which it was mingled during the
half hour in ice water, before being withdrawn
in order to be added to corpuscles d.) It could
 no longer act as a solvent on corpuscles d when
 mixed with them and placed in the conditions which
 produced solution in Experiment A of October 1st.

II. a + b on the other hand gave haemolysis in 16
 hours at 37° C. No further ice water stage required.
 Serum a having lost its power to dissolve
 corpuscles d the assumption is that it had given
 up the dissolving substance to corpuscles b when
 standing in the ice water previous to separation.
 This experiment confirms the assumption already
 stated, so that haemolysis now takes place by
 simply placing a + b in the incubator.

III./

III. Salt solution + b. No haemolysis after 24 hours in incubator. The presence of serum a which had not dissolved corpuscles d was therefore still a necessary factor for the solution of corpuscles b although the latter have been shown in Experiment II to have already absorbed an active substance from serum a.

There must therefore be two components in the toxine. One of these is absorbed by the corpuscles in the cold, but no solution of the corpuscles takes place unless the corpuscles are allowed to remain in contact with serum a during the incubator stage also.

October 15th.

		Incubator.	Ice water $\frac{1}{2}$ hour then Incubator.
1	Case T.S. blood oxalate mixture	000 **	+++ **
2	" " " inactivated serum + W. B. C. of same.	-	000
3	" " " inactivated serum + W. B. C. of same + Complement (my serum) <u>The complement was added</u> <u>after the tube was re-</u> <u>moved from the ice water</u>	-	+++
4.	" " " active serum + my W.B.C.	000	++
5.	" <u>writer</u> active serum of Case (i.e. complement) + W. B. C. of case T.S.	000	000
6.	" " W.B.C. in oxalate solu- tion.	000	000
7	" " Blood oxalate mixture	000	000

* W.B.C. = washed red blood corpuscles.

** 0 = No Haemolysis; + = Haemolysis.

The positive results did not show till observation was made at end of 24 hours. The negative results were still negative at end of 48 hours. takes place by the action of a substance These Experiments confirm that haemolysis in the cold combined with the action of another substance at a later period and at the blood temperature. The latter substance is also present in normal non-haemolytic serum.

Experiment 3 shows that the substance which acts during the incubator stage is the thermo-labile complement. The thermostabile amboceptor or Intermediary body must therefore be the substance whose affinity for the corpuscles exists during the cold stage.

The foregoing experiments prove isolytic as well as autolytic action. It is also shown that the corpuscles of T.S. behave normally when mixed with normal serum and placed in ice water and thereafter in the incubator.

Experiment with blood serum obtained during Paroxysm.

October 7th 1904. Case T.S. Beginning of a sharp paroxysm. At the time when blood was obtained the patient was feeling very cold in the hands and feet/

feet, he yawned occasionally and the nose and extremities of the limbs were somewhat cyanosed. Following the abstraction of blood, the symptoms developed rapidly. There were feelings of sickness breathlessness and giddiness. The urine, passed on the first occasion thereafter, had the colour of stout. The blood obtained was mixed gently with equal parts of the sterile oxalate^{-nacl} solution (as in the majority of the preceeding experiments).

A. A portion was immediately placed in the incubator and a decided haemolysis occurred in 24 hours.

One concludes that the amboceptor had already united with the cell before the abstraction of blood as haemolysis occurred without employing the ice water stage in vitro.

Another portion of the original blood oxalate mixture was kept warm and centrifuged quickly. The serum oxalate mixture was drawn off in a sterile pipette. It separated clear, so that there was no apparent haemolysis. The sediment of ^{in sterile warm salt solution (.85%)} corpuscles was washed and centrifuged. This was done several times. My own blood oxalate mixture was treated similarly, the serum and corpuscles being thus separated.

B. /

B. My serum (i.e. complement) + W.B.C. of T.S.

In incubator Haemolysis same as A.

This experiment also indicates that the corpuscles of T.S. had already taken up Intermediary Body before abstraction of the blood from the body. All that was necessary for solution therefore was the presence of normal complement and the maintenance of the temperature.

C. Same as B except that the preparation was placed for 40 minutes in ice water before being placed in the incubator.

No haemolysis in 24 hours.

An anomalous result which I am not able to explain.

The inadvertently long exposure to the action of cold may have so much delayed the return of activity in the complement that Intermediary Body had time to become unlocked from the cells.

D. Serum of T.S. + my W.B.C. Incubator only.

No haemolysis in 24 hours.

E. Same as D. Ice water $\frac{1}{2}$ hour and incubator.

Haemolysis occurred in less than 20 hours.

Experiment E proves that the serum of T.S. still contained Intermediary Body although experiments A and B showed that his corpuscles had absorbed sufficient to become haemolysed when supplied with complement.

The/

The limitation of the paroxysm may therefore not be determined by the using up of the Intermediary Bodies nor even by the formation of anti-intermediary bodies, but simply by a restoration of the body to temperature conditions which do not further favour the anchoring of Intermediary Body to the cells.

This single experiment however does not prove but merely suggests this idea.

Other experiments and controls were also prepared on this date, but unfortunately met with disaster. This is the more to be regretted as the opportunity has not again occurred of experimenting with the serum and corpuscles of the paroxysm.

Experiments with Blister Serum. Interval period.
October 22nd. T.S. in Hospital. Serum obtained from blister applied after cleaning of skin and application of a boracic fomentation. Surface of the blister was carefully washed with boiled water before being opened.

Serum was stored in a dry sterile glass tube.

Blood was also obtained from the patient and from the writer and the blister serum was mixed with the unchanged blood or with 5% suspension of the washed blood corpuscles as the table indicates.

(1)/

(1)	P.H. active serum	+	P.H. blood	{ice water 1/2 hour & 37° C.	+++
(2)	" "	"	"	+ " " "	- " 000
(3)	" "	"	"	+ My " ice water	" ++
(4)	" "	"	"	+ " " "	- " 000
(5)	" "	"	"	+ P.H.washed corpuscles ice water	" ++
(6)	" "	"	"	+ My " ice water	" +++
(7)	" "	"	"	+ " " "	- " 000
(8)	" "inactivated"	+	P.H. "	ice water	" 000
(9)	" "	"	"	+ My blood ice water	" 000
(10)	" "	"	"	+ My washed corpuscles ice water	" 000

The positive results on this occasion were obtained with remarkable rapidity. In less than one hour at the incubator temperature the serum in those tubes, which had been previously placed in the ice water, was deeply coloured. The haemolysis became complete. Those tubes which gave negative results were still negative at the end of eight hours.

No controls with normal serum were made on this occasion. All preceding and subsequent experiments indicate very well that no such haemolytic action is possessed by normal blister serum. Experiments that are represented in the above table, demonstrate that blister of T.S. serum, has haemolytic properties identical in nature with/

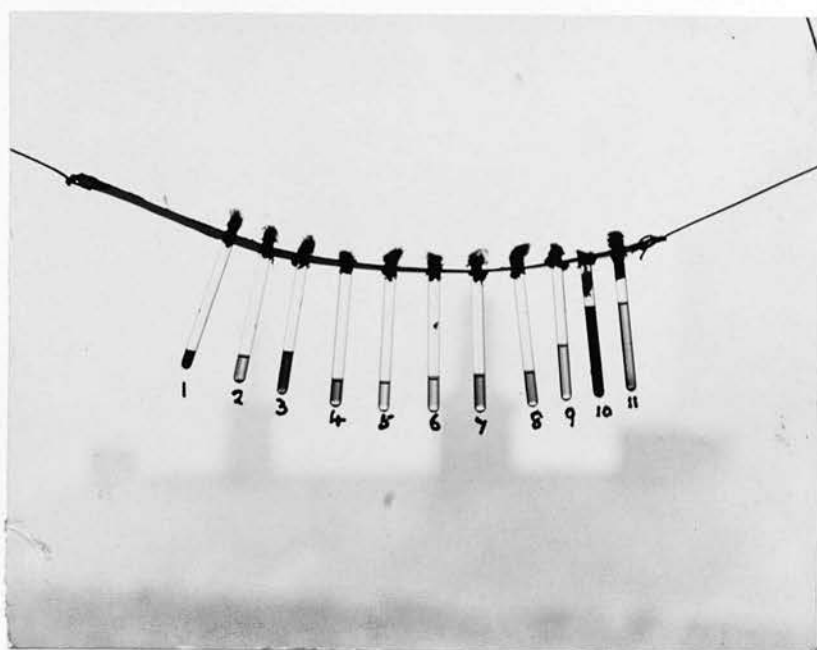
with that of the blood serum of T.S.

It would appear that the toxic action of the blister serum is at least as great as that of the blood serum.

October 26th.

The following series of experiments made with the blister serum of T.S. prove that the action of the blister serum is identical with that of the blood serum and with the mode of action of Immune sera as described by Ehrlich and Morgenroth.

P.T.O.



(1)	Active Serum of T.S.	+	my whole blood	(ice water & incubator)	+++
(2)	"	"	" " " + " " "	(incubator)	000
(3)	"	"	" " " + my washed corpuscles	(ice water & incubator)	++
(4)	"	"	" " " + " " "	(incubator)	000
(5)	"	"	"Control [*] + " whole blood	(ice water & incubator)	000
(6)	"	"	" " + " " "	(incubator)	000
(7)	"	"	" " + my washed corpuscles	(ice water & incubator)	000
(8)	"	"	" " + " " "	(incubator)	000

(9)(a) Serum of T.S. centrifuged from a preparation indential to No.3

at the termination of the ice water stage. (The serum is there-
fore assumed to have given up its amboceptors to the corpuscles
from which it is centrifuged) + (b) complement (i.e. serum of
control above shown to contain no haemolytic amboceptors +
(c) my washed corpuscles (ice and incubator)

000

The assumption that the amboceptors of T.S.'s serum were given
up to the corpuscles during the ice water period in No.3 is
therefore correct as the negative result now obtained shows
that a contained practically no amboceptors.

(10) Complement (same as b in (9)) + corpuscles to which a of 9 is
assumed to have given up amboceptors before being centrifuged
from the serum (incubator only)

+++

The assumption that the corpuscles take up the amboceptors during
the cold stage is correct and therefore when complement^{is} added to
the corpuscles it is now only necessary to put the preparation in
the incubator in order to get a pronounced haemolysis.

(11) Inactivated serum of T.S. + corpuscles as in (10) (incubator only)

000

* Normal serum, complement.

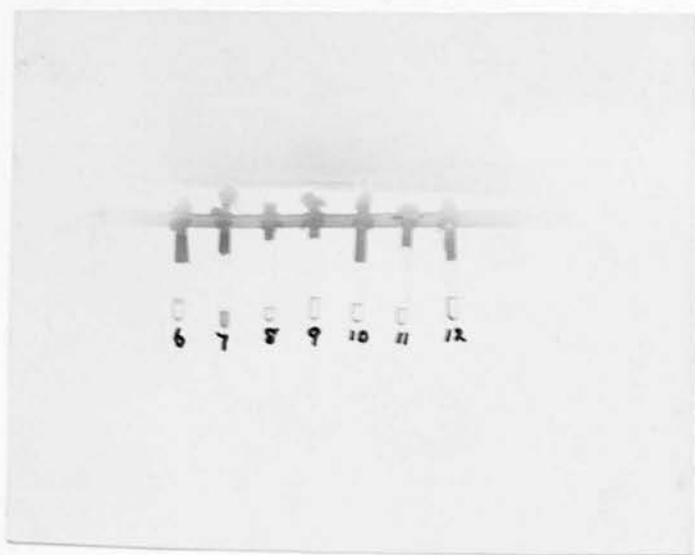
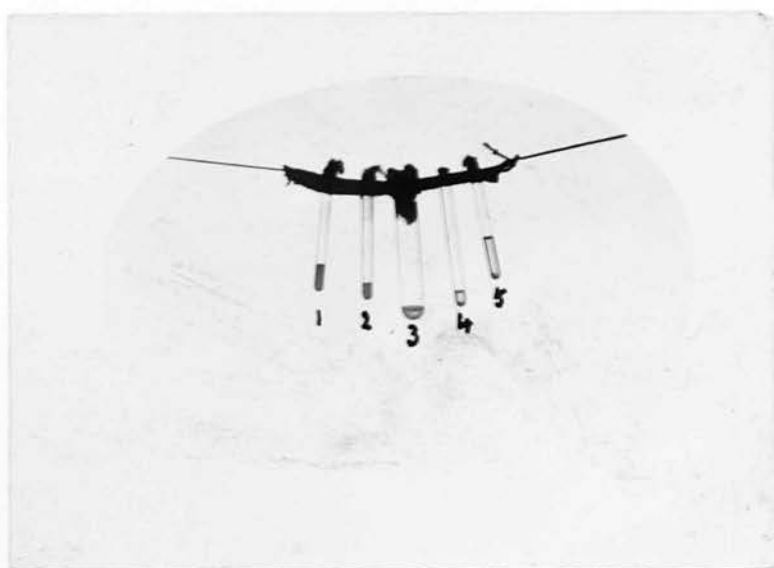
Although these corpuscles were loaded with amboceptors as the experiment 10 showed, the addition of the morbid serum containing amboceptors but no complement did not effect any haemolysis. When haemolysis occurs it is therefore by means of a haemolysin composed of two bodies, one of which is rendered inactive by raising to 56° centigrade for $\frac{1}{2}$ hour and the other is thermo stabile. The latter joins to the cells during the cold stage and as warmth is restored the thermo labile substance is enabled to act on the cell (probably by joining with the amboceptor or Intermediary Body.)

The photograph shows the results above described.

The negative results were still negative at the end of two days.

February 19th 1905. Case of J.M. (in Ward 26, Royal Infirmary, Edinburgh). Permission for this experiment was kindly granted by Professor Sir Thomas Fraser.

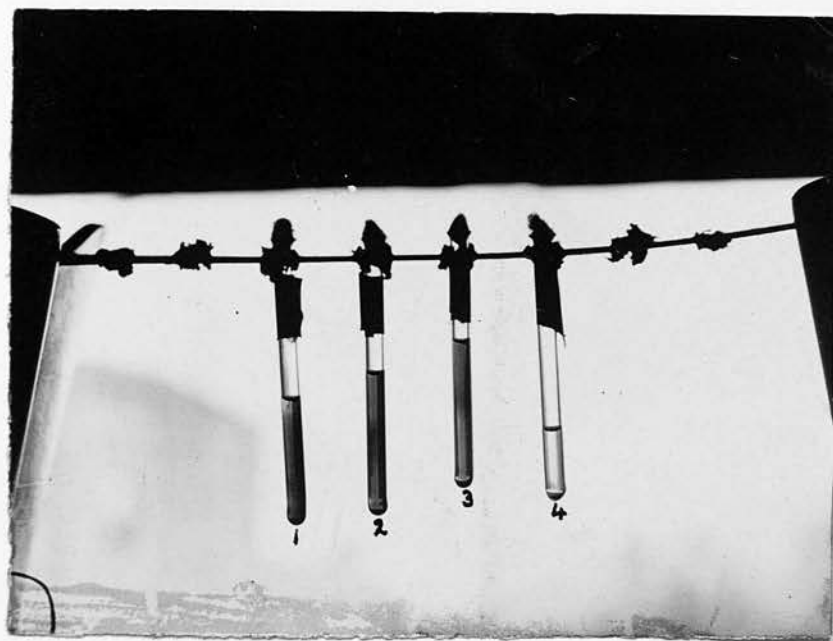
Blister serum obtained in usual way.



(1)	.2 cc serum + .2 cc my washed corpuscles	(ice water & incubator)	+++
(2)	" " " + " " " " "	(ice water & incubator)	+++
(3)	My washed corpuscles	(ice water & incubator)	000
(4)	.2 cc active serum + .2 cc my washed corpuscles	(Incubator)	000
(5)	" " " " + " " " " "	(Incubator)	000
(6)	" " inactivated + " " " " "	(ice water & incubator)	000
	serum		
(7)	" " " " {+ " " " " "	(ice water & incubator)	+++
	+ " " complement		
(8)	" " " " {+ " " my washed corpuscles	(Incubator)	000
	+ " " complement		
(9)	" " my washed corpuscles		
	+ " " complement	(ice water & incubator)	000
(10)	" " my washed corpuscles		
	+ " " complement	(Incubator)	
(11)	same as (3)	(ice water & incubator)	000
(12)	same as (3)	(Incubator)	000

Positive results obtained in three hours. The Photograph represents the results very well. (Exception to this remark must be in regard to No.3 [large tube]. The level of the fluid in this tube was so low that the concave surface of the fluid and the convexity of the lower end of the tube have caused the fluid to appear dark while it was absolutely colourless.)

The history of this case was published by Sir Thomas Fraser in the Edinburgh Medical Journal of 1897 page 313. It is there stated that J.M. had not suffered previously from ague fever or syphilis. His wife had not had any miscarriages. It has since been learned that J.M. previously suffered from Malaria and at the present time he has an aneurism of the aorta.



The following experiment was done with the object of determining if prolonged exposure of the amboceptors or complement to cold could hinder or prevent haemolysis.

- I. .2 c.c. normal complement was placed in a tube in ice water for $2\frac{1}{2}$ hours. When this time had elapsed .2 c.c. inactivated serum of T.S. and .2 c.c. of 5% normal washed corpuscles in normal salt solution were added. The tube was kept in the ice water for 30 minutes longer. It was then placed in the incubator. Haemolysis occurred very quickly and completely.
- II. .2 c.c. inactivated serum of T.S. was likewise kept in ice water for $2\frac{1}{2}$ hours. Then .2 c.c. of the W.B.C. were added and the ice water stage was continued for 30 minutes longer. Finally .2 c.c. normal complement were added and the tube was then immediately placed in the incubator. Haemolysis same as above.
- III. Same as ^{No. I} except that the normal serum was exposed to the ice temperature for only $\frac{1}{2}$ hour before adding the inactivated toxic serum and normal corpuscles. Haemolysis same as above.
- IV. .2 c.c. normal serum (complement) + 1 c.c. normal W.B.C. Ice water $\frac{1}{2}$ hour. Incubator. No haemolysis.

The photographs represent the condition of the preparations after they had been kept for three hours in the incubator.

It appears from these experiments that both complement and amboceptor may suffer prolonged exposure to cold without interfering with their haemolytic action.

These recent experiments entirely confirm the experiments I had performed in 1903 and those of Donath and Landsteiner in 1904, and they appeared to me to afford ample evidence for the conclusions which I already arrived at.

One of the theories on which this research has been based is thus I venture to maintain not only proved but amplified. The theory may be briefly stated as follows :-

- (1) That there is present in the body a potential toxine which becomes active only under certain favouring conditions, one of which is exposure of the blood to cold.
- (2) That the potential toxine is composed of two bodies one of which possesses the characteristics of an amboceptor and the other those of complement.
- (3) That the activity of the toxine, which is promoted by cold, is due to the special characteristics of the amboceptor.

Paroxysms are thus caused, not as some maintain by/

by the development of a toxic substance during the exposure to cold but by the toxine, always potentially present in affected individuals, becoming active during such exposure.

The experiments also give evidence of much variation in the toxicity of the serum especially of the blister serum.

There are three possible explanations of the variation in the degree of toxicity.

Either (1) The available free amboceptors in the serum may be diminished by a paroxysm.

or (2) There is anti-amboceptor formation.

or (3) The supply of amboceptors from an undefined source varies in amount at different times.

Reference has ^{elsewhere} already been made to the part played by the leucocytes during Haemolysis in vitro. A great deal requires yet to be learned regarding this matter. In my experiments in vitro, there was much evidence that the leucocytes themselves underwent a rapid destructive process. Microscopic specimens, prepared from normal blood + the serum of T.S., frequently shewed this. But I will not venture to affirm that this destruction of Phagocytes occurs also in vivo during a paroxysm.

The literature lends little help to the elucidation of this subject. The condition of the leucocytes during paroxysms is a question which has been overshadowed by the idea which haemoglobinuria suggests most prominently, viz. destruction of red cells.

In a research entitled "Die morphologischen und tinktoriellen Veränderungen nekrobiotischer Blutzellen" Dr. K. Bödön⁽⁴⁰⁾ of Budapest has found that the order of destruction of cells is

- (1) large mono-nuclear (Ehrlich)
- (2) large lymphocytes

(b) polynuclear. The neutrophile are more

resisting than the acidophile.

- (3) Small lymphocytes.
- (4) Erythrocytes.

(41)

Silbermann thought that the worst symptoms of Paroxysmal Haemoglobinuria were not due to the destruction of the red cells, but were due rather to the destruction of the white cells, or to a change in them which caused the production of an enormous amount of fibrin ferment. To elucidate this point, estimations were made in the case of T.S. by the Resident Physician at Leith Hospital. These estimations were made immediately preceding and immediately following a paroxysm. The number of white cells was less after the paroxysm (see p.25)

Mattirolo and Tedeschi (loc.cit. page 262) found the number of white cells reduced during the paroxysm. In their case there was a leucocytosis preceding the paroxysm, followed by a reduction during it. Another instance in which a blood count was made immediately preceding and during a paroxysm was in a case of Donath's (loc.cit. page 10) In this instance also there was a reduction of leucocytes as the result of paroxysm.

(42)

In the case published by Sir Thomas Fraser the enumeration of the leucocytes has been done in a more thorough manner than I find in any other record.

The /

The enumerations were made simultaneously but independently by two observers at ten different periods in the course of 27 hours. Before the beginning of the paroxysm the leucocytes were 15,000 per c.m. and during the paroxysm the maximum reached was 24,000, so that this observation is not in agreement with those of Mattiolo and Tedeschi, Silbermann and Donath.

From my own observations in vitro in which I have found an extreme degree of phagocytic activity (Ruziczka phenomenon) I would expect that some degree of leucocytosis exists during the paroxysm.

In my earliest experiments, conducted in 1903* I have described the action of the serum of T.S. on the fibrin formation of my own and other normal blood. I indicated that the fibrin was very light and fragile, and did not contract like fibrin in normal serum. To this must be added an observation (which I have frequently made since then) in regard to the fibrin formation of the blister serum itself. viz:-

The coagulum was always much less copious than that of normal serum, and sometimes almost entirely absent.

To/

*Edinburgh Medical Journal
Dec. 1908*

To decide if the serum of T.S. could dissolve normal fibrin I did an experiment as follows :-

Blister serum of T.S. + fibrin of normal serum	37° C
	for 24
	hours
	<u>No solution</u>
	<u>of fibrin.</u>

"	"	" " " +	"	"	"	serum in ice
						water for
						half an
						hour and
						then 37° C
						for 24
						hours.
						<u>No solution</u>
						<u>of fibrin.</u>

Having concluded this description of the experiments which have been performed the all important question arises as to what this haemolysin substance is, and how it comes to be present. Ehrlich and Morgenroth failed to produce an autolysin in their experiments on animals, but we know that the body is constantly elaborating substances of a toxic nature, and if the equilibrium of production and excretion is disturbed, auto intoxications occur which are still but little understood. Moreover past failure

failure to produce an autolysin experimentally cannot be accepted as a final settlement of this question. (43)

TRAUMATIC HAEMOGLOBINURIA.

In attempting to discover how the autolysin comes to be present in individuals affected with Paroxysmal Haemoglobinuria, some important points are suggested by the consideration of Haemoglobinuria resulting from extravasation of blood, and the formation of haematomata after trauma. What does pathology teach us regarding the sequelae of such extravasations?

The problem as to whether the leucocytes are (in the original sense of Metchnikoff) the sole or even the overwhelming factor in the removal of a haematoma, or whether there is also some other dissolving process proceeding simultaneously by virtue of the serum (extracellular lysis) is beset with difficulties. There is information at hand which permits one to surmise that the process is no mere matter of ingestion and transportation by phagocytic action.

Firstly, cases are recorded carefully which shew that haemoglobinuria may result from an extravasation of blood traumatic or otherwise.

(43)

Thus Leonar Michaelis records a case in which there was much extravasation of blood into the general peritoneal cavity. Haemoglobinuria occurred twice during the absorption of this.

Discussing the manner in which Haemoglobinuria resulted/

resulted, he considers it probable that both of two possible causes were in action. Firstly the Haemoglobin, which had been absorbed unchanged, contributed; and secondly, there was the occurrence of haemolysis which caused destruction of the patients own blood. His view will be discussed more fully immediately.

At the meeting of the Royal Medical and Chirurgical Society on March 24th 1903 a case of haemoglobinuria of traumatic origin was described by C.W.Ensor and I.O.Wakelin Barratt.⁽⁴⁴⁾

The subject was an asylum patient who was accustomed to beat his forehead with his right hand with such violence as to produce a "sickening thud." He would continue to do this for half an hour or longer at a time. Such bouts of extreme violence were quickly followed by haemoglobinuria. There was no other exciting cause evident, and each attack had been preceded by this violence. ~~///~~ The violence was so great that extravasation of blood must (in the authors' opinion) certainly have occurred, and yet there was never any visible bruising. It was therefore assumed that the/

the local injury had set free a haemolysin which destroyed the red blood corpuscles and set free haemoglobin in the plasma. It was suggested in the discussion, which followed that the liberation of the haemoglobin was probably not the result of the action of a haemolysin but the result of the excessive mechanical vibration, as occurs in centrifuging blood. (I have never observed that haemoglobin was set free by this process, when properly performed with fresh blood). There was no special fragility of the blood corpuscles. The more general opinion of the members who spoke was that the blood destruction depended on the action of a locally produced haemolysin. Injury acted as the exciting cause just as cold or violent muscular exertion might in predisposed subjects. Ehrlich's view (already quoted) that in paroxysmal haemoglobinuria a local haemolysin might be developed by the epithelial cells of the blood vessels was quoted as having significance in relation to this particular case.

(45)

Dr. Karl Kober reports a case of a woman aged 50, who had torsion of the pedicle of an ovarian tumour, accompanied by Haemoglobinuria. In the centrifuged sediment of the urine no corpuscles, kidney/

kidney epithelium, or tube cases were observed. Operation confirmed the diagnosis of torsion. The tumour had very thick walls which were infiltrated with blood. The fluid contained in the tumour was a haemorrhagic pseudo-mucinous fluid. On the day following operation there was still slight albuminuria, but no haemoglobinuria. The albuminuria disappeared 24 hours later. The patient was discharged three weeks afterwards as cured. Kober believes that the great torsion of the short pedicle pressed the blood and other matter contained in its cavity into the walls of the cyst. The haemoglobin, thus expressed, was set free in the circulation and the quantity was too great for the liver to convert into bile pigments. The haemoglobin was consequently passed out in the urine. He notes that Michaelis had a different explanation for the causation of Haemoglobinuria in his case.

(46)

Tauber has described a case of ruptured tubal gestation with haemoglobinuria. The case presented the classical symptoms. No operation was performed on the patient, who was treated with opium and camphor. Three days after the sudden onset, dark red urine was passed containing a few "shadow" red cells. The/

The patient recovered quickly from the attack. There was blood in the urine for 2 days more and then the urine became clear and free from albumin and blood. Between the period when she was first examined and the date when haemoglobinuria occurred, the dullness of the abdomen had become diminished. Several weeks later when a pelvic examination was made a haematocoele could be diagnosed.

(47)

William Muir has recently reported a case that seems worth noting at some length.

A farmer aged 20 was knocked down by a boar. A severe wound (through two thicknesses of clothing) was received on the outside of the right knee and a small fragment from the head of the fibula was broken off. The wound was immediately attended to and after careful cleansing was completely closed. The patient was confined to bed. The urine of the same evening contained haemoglobin and was almost black. This condition of the urine persisted for several days. The leg was considerably ecchymosed and there was a large effusion into the right knee joint. No carbolic acid was used in dressing the wound. Steady progress was made for a month when the patient was attacked with Influenza/

Influenza. On the following day there was again haemoglobinuria. $3\frac{1}{2}$ years previously while engaged carrying a gun and heavy gamebag for the greater part of the day, he perspired freely as the result of severe physical exertion and a blood colour appeared in his urine, persisting for several days. 18 months later he was revaccinated, being at the time in perfect health. A day or two after the vaccination his urine had the colour of blood and shewed a large deposit of urates and albumin. Muir points out that two of the patient's attacks followed traumatism, one succeeded severe physical exertion, and one accompanied an attack of febrile catarrh. The general health was on all occasions exceptionally good.

In the earlier literature several instances are to be found in which trauma was the precursor of haemoglobinuria.

(48) (49) (50)
Davis, Day and Socoloff have published such cases, which were true Paroxysmal Haemoglobinurias.

Haemoglobinuria therefore may be the sequel of an extravasation of blood or of an intra peritoneal haemorrhage. How can this occurrence of haemoglobinuria/

haemoglobinuria be explained in view of ⁽⁵¹⁾ Von Jaksch's opinion that 'absorbed' haemoglobin is not excreted as such, but as urobilin? If Von Jaksch's opinion regarding 'absorbed' haemoglobin is accurate then Michaelis' theory that the traumatic haemoglobinurias result from the action of an auto-haemolysin on the 'circulating' blood seems the most probable explanation.

A point of the utmost importance is Camus' experimental proof that the destruction of $\frac{1}{57}$ of the total blood of the body is necessary in order to produce haemoglobinuria. It is difficult to believe that this amount of blood extravasation occurred in all of the above cases. But if this amount was not extravasated, haemoglobinuria could not result from the absorption of the haematoma. Those individuals had undoubtedly received an intra-peritoneal or subcutaneous injection of their own blood as the result of an injury viz. rupture of tubal pregnancy, etc. What process destroys the corpuscles in their new situation and liberates the haemoglobin so that $\frac{1}{57}$ of the total globular haemoglobin in the body may at some period be circulating free in the plasma and begin to be excreted in the urine?

In/

In the majority of my experiments with various human sera and corpuscles, I have observed that the serum remained clear for 2 days and in some cases for as many as five days. Indeed, corpuscles in .85% NaCl solution do not remain intact so long as in normal human serum. In the salt solution I found that 42 hours was the average period at which coloration of the fluid occurred. What then is the active process in these traumatic haemoglobinurias?

I have already quoted experiments which indicate the extremely active phagocytosis of the red cells which follows on injection of an immune serum.

Levaditi* has gone so far as to found a theory of haemoglobinuria on the fact of this energetic Phagocytosis of red cells loaded with Intermediary Body. According to him, haemoglobinuria occurs because corpuscles loaded with Intermediary Bodies are very eagerly absorbed by the Phagocytes, in whose interior solution then follows; and the haemoglobin which is excreted in the urine, has been previously carried to the kidneys by the leucocytes which then give it up. He supports this opinion by experiments which were devised on the assumption that the injection of Bouillon into the Peritoneal Cavity of animals, antecedent to the injection of a haemolytic serum, stops any extracellular solution (i.e.)

* Loc.cit.

(i.e. by complement) of the red corpuscles. In spite of this, however Phagocytosis is still more energetic and haemoglobinuria occurs specially soon. Gruber and Ruziczka each did extensive experiments to test Levaditi's conclusions and the assumption regarding the action of Bouillon on which his conclusions are based.

Thus Gruber* injected (a) normal corpuscles of fowls and (b) corpuscles of fowls containing Intermediary Bodies into the peritoneal cavity of

- (I) normal guinea pigs
- (II) guinea pigs which had Bouillon previously injected
- (III) guinea pigs which had fowls corpuscles previously injected.
- (IV) guinea pigs injected previously with fowl corpuscles and then with Bouillon.

The fate of the corpuscles was ascertained from samples obtained by means of capillary tubes, and finally by observations made after killing the animal. As already indicated, Gruber agrees with Levaditi in maintaining that erythrocytes containing Intermediary Body undergo Phagocytosis much more quickly and in much greater number than do normal erythrocytes.

Gruber also found that leucocytes which have been previously treated with a haemolytic action act

* Loc.cit.

act specially energetically and that Bouillon intensifies the phagocytosis still more.

Gruber however denies that Bouillon injection hinders the extra-cellular solution of the corpuscles. He considers this theory as erroneous as Metchnikoff's theory that Pfeiffer's phenomenon of Bacteriolysis could be stopped by the injection of Bouillon. He maintains that Levaditi was consequently wrong in believing that the haemoglobinuria he obtained could not result from the action of the serum but was the result of phagocytic action alone. Gruber believes that the Bouillon, by increasing the activity of the phagocytes, merely excludes a larger number of corpuscles from the action of the Haemolytic fluid. The solution by the phagocytes and by alexin (complement) are entirely different processes. The phagocytic (i.e. intracellular) process is an extremely slow one - much slower than the extra cellular (by complement). None of his observations, Gruber states, indicated that the alexin required for the extra cellular process was derived from the leucocytes, although the two processes Lysis and Phagocytosis generally proceed simultaneously.

Gruber maintains that the changes in the corpuscles/

corpuscles which are absorbed by the phagocytes are only in a slight degree similar to those produced by alexin. In vitro one can see that the entire blood cell and a great bulk of the haemoglobin becomes fully digested and destroyed in the interior of the phagocytes. There is also no trace of free alexin demonstrable in the fluid in which the phagocytes have been placed, when tested with either normal or Intermediary Body-containing corpuscles, whether that fluid be inactive normal serum, or inactive immune serum, or salt solution. The free corpuscles remain intact when those taken up by the phagocytes have completely perished. Gruber is of opinion that a small proportion of the haemoglobin of the devoured corpuscles leaves the phagocytes without further breaking up. This however is always a small amount. To a small extent also the corpuscle swells up in the interior of the phagocyte. Those who have maintained that the alexin or complement is produced by the leucocytes have called it a Macrocytase* and have denied that it can be formed from the microphages. But Gruber holds that intracellular lysis occurs as often in microphages as macrophages, and therefore this process does not appear to be dependent on the action of Macrocytase alone.

* Metchnikoff (52)

Gruber concludes that his observations thoroughly disprove

- (1) that phagocytosis plays a noteworthy role in originating haemoglobinuria:
- (2) that Levaditi's observations in any way prejudice this conclusion.

He does not contest Levaditi's opinion that Intermediary Body is a promoter of phagocytosis, but he believes that both processes, Lysis and Phagocytosis, are important weapons of the organism.

Gruber believes that the haemoglobin, which is not taken up by the active phagocytosis, is the cause of haemoglobinuria.

Now there is no reason for believing that the phagocytosis in the above mentioned traumatic cases was in any degree more energetic than that occurring in the experiments which Gruber performed. It may therefore be concluded that haemoglobinuria as the sequel of a haematoma does not result from the incorporation, solution and transportation of the erythrocytes within the leucocytes to the kidneys.

It appears from Gruber's experiments that the amount of Haemoglobin necessary to produce haemoglobinuria ¹_(57 of the total in the body) could not at any given period be circulating free in the plasma as the result/

result of phagocytic action upon the haematoma.

Gruber moreover found that the haemoglobin of the ingested red cells was completely destroyed by them. That which is not taken up is, according to Gruber, the portion excreted in the urine. If Gruber's conclusions are correct, the body must therefore possess some more rapid means of dealing with such extravasations than the most energetic phagocytosis could accomplish.

How then can the phenomenon of haemoglobinuria in the cases quoted be otherwise accounted for?

I venture to suggest that certain classical investigations provide a basis of fact upon which one may reasonably found an explanation of the condition under consideration.

It has been already stated that Ehrlich and Morgenroth^{*} have demonstrated hetero and isoclysis but were unable to produce autolysis by the same methods. If the researches of Ehrlich and Morgenroth are to be accepted, then traumatic haemoglobinuria cannot be explained by the action of an auto-haemolysin. There are however many reasons for questioning the finality of their conclusion in this particular respect. Gruber^{**} for instance has stated that

Antibody/

* Loc. cit.

** Loc. cit.

Antibody formation is a secretion or substance produced by "irritation." Again he says that the formation of Antibody is in no way dependent on the poisonous properties of the Antigene (i.e. a substance which by "irritating" the organism causes the formation of an antibody.) An antigene may chance to be poisonous, and a poison may have the property of being an Antigene.

Metchnikoff (loc.cit page 741) says
 "J'ai émis l'idée qu'on pouvait obtenir des sérums contre toutes sortes d'éléments cellulaires, et je me suis mis, avec mes élèves, à réaliser cette hypothèse."

(52a)

Metchnikoff working in Metchnikoff's laboratory obtained an antibody to spermatozoa by injecting spermatozoa subcutaneously into the animal from which they were obtained. That is to say he obtained an auto-spermatotoxine. Pfeiffer and Friedberger (53) injected cholera Immune Bodies and obtained cholera Anti Immune Bodies, although the Immune Bodies are poisonous only to Cholera Vibrios. Therefore (1) the formation of antibodies is a biological process which is not necessarily connected with any poisonous action of the Antigene as the above statements show; and (2) Antibody may be formed in an individual when
 a/

a normal product of his body is transferred to an unusual position. Normal products of the body so transferred thus become an Antigene. (3) The rôle of Antibody formation can be assumed by tissues having no immediate relation, either in position or function, to the organ from which the antigene was obtained.

There is therefore a basis of fact on which to found an explanation of these traumatic haemoglobinurias. It becomes apparent that Michaelis' assumption, that there was an autolysin produced in his case (p.96) is a reasonable one. His arguments, however, are not those that I myself have just used. He contents himself with stating that Ehrlich's theory is comprehensive enough to explain the production of an autolysin, in spite of the abortive efforts of Ehrlich and Morgenroth to produce such a body.

Michaelis proceeds to explain the reason why autolysin is not constantly formed in the blood. This, he says, is because the side chain or group of the red cell is not in a soluble form, and therefore cannot react on the receptor. According to Michaelis the side chains, during absorption of a blood effusion, are in a soluble form and combination/

combination with the receptors takes place, and receptors are formed in excess, thus constituting an autolysin. This again causes the formation of an antiautolysin which generally prevents an autolysis. If the absorption of blood effusion is so quick that an antiautolysin has not time to form in sufficient quantity then destruction of blood corpuscles of the individual occurs. Michaelis believes that is what occurred in his case.

Critique. I have already indicated facts (pp 108 & 109) which render the assumption regarding an autolysin a reasonable one, but the negative results of Ehrlich and Morgenroth have to be kept in mind. It appears to me that Michaelis is scarcely justified in assuming that autolysin is not constantly present in the blood and yet he proceeds to explain why it is not there. The autolysin may possibly be a product of metabolism which is present normally in small amount (Gruber)* That the side chains are not in a soluble form in circulating blood (but become so during absorption of a blood effusion) is an ingenious assumption, which however fails to explain how the solubility is produced. That is fundamentally important.

As already/

* Loc. cit.

As already indicated Michaelis says the haemoglobinuria probably resulted (1) in consequence of the absorption of unchanged haemoglobin from the intra peritoneal haemorrhage, and (2) by the action of an auto lysin on the individual's normal blood corpuscles, when sufficient time had not elapsed to permit the formation of antiautolysin.

In addition to the objection I have already urged against Michaelis' views, I consider that the rapid absorption of the haemoglobin of the effused blood must have been preceded by solution of red cells. But the process by which solution was effected is not explained. The conservative nature of normal serum for corpuscles must be borne in mind (page 103). Moreover the haemoglobin, which is absorbed, is not excreted as such according to Von Jaksch. Perhaps it would be still more correct to say that haemoglobin which is absorbed by the leucocytes, is not excreted as such. (Gruber*)

Regarding the second factor which Michaelis mentions the facts of the case which he publishes appear to be condemnatory of his views on anti-autolysin formation. For in his case the haemoglobinuria appeared on the day after the onset of the illness and lasted throughout next day. On the 5th/

*Loc.cit.

5th day after its disappearance it reappeared much exaggerated and lasted for 4 days, the third day being the worst. Such a history surely indicates that there was a sufficient opportunity for the formation of an anti-auto-lysin between the first and second attacks to have prevented the latter of these.

(54)
It must be said however that Besredtka has demonstrated the presence of an anti-auto haemolysin in human serum and he says the failure to demonstrate an auto haemolysin may be in consequence of the former neutralising the latter.

Kober's view regarding his case is that the torsion of the pedicle squeezed blood from the contents into the wall of the cyst. He assumes that thus haemoglobin was set free in the circulation and its amount proved too great for the liver to transform. How the haemoglobin was set free is not explained, as he only states that the walls were infiltrated with blood corpuscles. It is difficult to imagine that there was a quantity of free and pure haemoglobin in the cyst originally equal to $\frac{1}{57}$ of the total in the blood. Tauber's views are similar to those of Michaelis.

Consideration of the facts recorded in these traumatic/

traumatic cases has suggested the question May not the corpuscular elements of the blood effused (e.g. into the peritoneal cavity) act as an Antigene in contact with the peritoneal lining? For to this membrane the effused blood is really a foreign substance i.e. stagnant blood tending to coagulate. Therefore in accordance with Metalnikoff's experiments on spermatozoa, it appears that in an intra peritoneal effusion an antibody may be formed which co-operates with the alexin to dissolve the cells and only then may absorption of Haemoglobin rapidly take place. Phagocytic action of course also plays its part in the absorption of the blood, but not (according to Gruber) in the production of haemoglobinuria. The haemoglobinuria, I venture to suggest, results from the action of an antibody which may not only dissolve the haematoma, but may also act on the circulating blood. The portion removed by the phagocytes probably undergoes the changes within these cells described by Gruber. The portion absorbed after solution by an autolysin may not be transformed into urobilin (i.e. the statement of Von Jaksch may not be applicable under such conditions,) but with our present knowledge no useful purpose will be served by entering on any further speculations/

4 80

speculations, as to the manner in which autolysin may originate in traumatic cases.

Is it probable that cases of paroxysmal haemoglobinuria may sometimes originate from antecedent occurrences such as are indicated in the above cases, i.e. trauma?

In spite of the importance of such a question it cannot be considered at any length because (1) the further history of such traumatic cases has seldom if ever been recorded. The only apparent exception is the case published by Muir (page 100) which shows that a paroxysm could be produced not only by trauma but also by exertion or febrile catarrh. This fact tends to bring these traumatic cases into a nearer relationship with those we recognise as paroxysmal. (2) Because the histories of paroxysmal haemoglobinuria cases have in the past been vague in regard to a matter (viz. a history of trauma) which did not appear to have any bearing on the etiology.

It would be well if histories of cases were explicit regarding injuries, blood extravasations etc. In the case of T.S. there was a history of bruise of the back requiring treatment in Hospital. This accident however occurred 21 years before the onset of the present malady. In the case of M.R. the mother/

mother attributes the onset of the illness to the many whippings given to her daughter at school.

Consideration of this 'specific' origin of Paroxysmal Haemoglobinuria cannot for the present be much further pursued, but attention may at least be drawn to the classical description of Lichtheim written many years ago, as it is somewhat suggestive of a similar idea. Lichtheim^{*} makes reference to Ponfick's work on the transfusion of lamb's blood into man, and he then suggests that destruction of corpuscles in this disease^{**} takes place in the blood in the same way -

"dass ein Theil derselben (i.e. the individuals corpuscles) in der Blutbahn ebenso wie die rother Blutkörperchen der fremden Species untergeht und ihren Farbstoff an das Blutserum abgiebt (p.1160)

He also compares the symptoms following such transfusion to those occurring in paroxysmal Haemoglobinuria e.g.- The shivering, rise of temperature and sweating. "Ja es geht die analogie so weit dass das Aufschliessen von Urticaria quaddeln, die unser erster Patient bei seinem ersten Anfall an sich beobachtet hat, auch gelegentlich als Effect der Lammblood transfusion beobachtet worden ist." (p.1162)

This analogy does not appear to have been chosen by /

* Loc.cit.

** Paroxysmal Haemoglobinuria

by Lichtheim for the sole purpose of indicating that the seat of the blood-destruction was in the general circulation for the entire similarity of the artificial Haemoglobinuria to the paroxysmal form was enlarged upon and emphasised. Whatever may have been the true meaning of Lichtheim however, the analogy is one which, in my opinion becomes more and more appropriate as our knowledge of Paroxysmal Haemoglobinuria increases. For in the blood and lymph of individuals affected with Paroxysmal Haemoglobinuria there appears to be present a substance which, under certain conditions, acts as an antibody or Immune body to their own blood corpuscles. Whether this toxine sometimes develops in consequence of an antecedent effusion of blood is at present difficult to decide but it is hardly probable that the toxine originates in this manner in all cases of Paroxysmal Haemoglobinuria. Continuing the consideration as to the source of the toxine it may be recalled that malaria was assigned an important rôle in the causation of Paroxysmal Haemoglobinuria by the earlier writers.

A history of malaria is sometimes obtainable as in the case published by Professor T.R.Fraser, Edinburgh, but the majority of cases have no such history (v.a. page 88)

Is/

Is there any other probable explanation for the presence of the toxine?

A history of syphilis is obtained in a large proportion of the cases. Curiously the first case of P.H. described was in a boy who shewed evidence of congenital syphilis.*

(55)

W. Stempel examined the literature of 77 well described cases and found that 29.8% of the cases had suffered from syphilis.

Donath (page 11 & 12) found that 3 cases were syphilitic out of 5 which came under his own observation. Of my own cases T.S. had previously suffered from syphilis and the history of M.R. was doubtful. Mrs.R. had four successive miscarriages following the birth of M.R.

(56)

Murri claimed to have cured two cases of Paroxysmal Haemoglobinuria by inunction of mercury.

Kopp⁽⁵⁷⁾ cured a case by inunction.

Authors are quite clear that syphilis cannot be traced in all cases viz. Boas (l.c.) Hayem, (l.c.) Carpenter,⁽⁵⁸⁾ Mattiolo and Tedeschi (l.c.) among others.

(59)

Rossoni tried the effect of cold on the blood of some syphilitic patients with negative results. With the object of determining whether or not the serum of syphilitic individuals had haemolytic properties/

*Dressler Loc.cit.

properties I did one experiment in a case of tertiary syphilis. The serum was mixed with normal blood corpuscles and the preparation was placed in ice water for $\frac{1}{2}$ hour and then in the incubator. No haemolysis occurred. The consideration given in the immediately preceding pages to the subject of Traumatic Haemoglobinurias, and of Malaria and Syphilis has been entered into for the purpose of obtaining, if possible, some idea as to the mode of origin of the toxic body in Paroxysmal Haemoglobinuria. The conclusions that may be drawn are as follows:-

1. While Haemoglobinuria sometimes results from an effusion of blood and cases of Paroxysmal Haemoglobinuria (Day's) are reported to have originated from previous trauma, sufficient data are not yet recorded to permit the causal relation of trauma to Paroxysmal Haemoglobinuria to be affirmed or denied.
2. While Malaria and syphilis are fairly frequent antecedents of Paroxysmal Haemoglobinuria the previous existence of either is not a necessary factor for the occurrence of this disease in an individual.

Such considerations have convinced me of the great importance of conducting a very full enquiry into the previous clinical history of cases as they occur.

I have not found that any bacteriological
examination of the blood of this disease has been
made beyond that which was limited to the
examination of films prepared directly from the
blood of the affected individual. The bacteriological
examination of the blood is worthy of a more
elaborate research than has yet been devoted to it.

THE METABOLISM IN PAROXYSMAL HAEMOGLOBINURIA.

I shall proceed now to quote briefly certain conclusions arrived at by Gruber (l.c.) regarding the nature of antibodies (Intermediary Bodies).

Antibodies are a secretion or product resulting from "irritation" but whether they are normal constituents of the body formed in excess or an entirely new substance is not accurately determined.

It is still possible to maintain that they are constituents which are normally present in very small amount, and it is known that normal blood serum contains a substance, which possess certain antitoxic agglutinating properties. This fact supports the view that Intermediary Bodies are normal metabolic products, yet it cannot be maintained that there is identity between this substance in normal blood and the Intermediary Body or Antibody of a specific serum. No fundamental difference in action has been proved between the normal and specific antibodies, and it is possible that all those countless antibodies may be, in small quantities, normal body constituents.

In consequence firstly, of the fact that there is almost complete ignorance regarding the chemical nature/

nature of Immune Bodies, and secondly, of my conclusion that Paroxysmal Haemoglobinuria is attributable to the activities of an Intermediary Body, (which is in fact an Immune body to corpuscles of the affected individual,) it becomes important to investigate the state of the metabolism in this disease. Thus information may be obtained regarding the action and perhaps also regarding the chemical nature of the toxic substance.

The intimate association of haemoglobinuria with conditions which affect the metabolism is particularly obvious in the case of horses. "The only predisposing condition known is overfeeding and idleness after work, as it seems to attack city work horses in high condition and stallions just after the breeding season. Indeed in many of the large American carriage and express stables it is known as "Monday morning disease" as it is most apt to appear on that day of the week after 36 hours' rest and high feeding (Woods Hutchison). Lucet (60) (61) has also pointed out this manner of onset and he thinks there is an accumulation of urea and extractive stuffs in the blood in consequence of chill causing congestion of the kidneys.

Adam/

(62)

Adam has recently indicated this weekly periodicity in man in connection with conditions supposed to be of toxic origin. He observed "that asthma, urticaria, albuminuria and epilepsy are not infrequently caused in certain people on resuming active work after a Sunday of ease and good feeding." It was particularly observed in those who had hard physical labour during the week,

(63)

Siedamgrotzky found that in the blood of a horse affected with Haemoglobinuria, ^{the} urea was 6 times increased. The excretion of the same and of the extractive stuffs in the urine was 5 times increased (but the hippuric acid was diminished.) He formed the opinion that the attacks were caused by accumulation of these substances in the blood. Numerous authors have compared the disease in man to that in the horse.

Camus insists on the great similarity of Haemoglobinuria in man and the horse in regard to cause, onset, the majority of the symptoms, evolution, duration, termination and recurrences. The muscular manifestations alone present differences, and the gravity of these symptoms in the horse may be due to a greater susceptibility of the muscular/

muscular tissue of this animal and to the fact that it is not able, in the same way as man, to remove the cause i.e. cold and fatigue (Loc.cit page 73).

Lichtheim in his classical description of Paroxysmal Haemoglobinuria likens it to the Haemoglobinuria of the horse particularly as regards the etiology (loc.cit. p.1167). In the case of T.S. (my case page 22) it is recorded that on one occasion the patient "completely lost the power of both legs for two or three hours." It is not stated if this occurrence synchronised with a paroxysm.

I am not aware that the literature contains any record of an occurrence so identical to this very prominent symptom of Haemoglobinuria in the horse.

The significance of an established identity between the disease in man and the horse is that the investigation of the condition of the state of the metabolism in man becomes more interesting and important.

(64)

Bollinger reports a case in which haemoglobinuria occurred only after prolonged exercise on deficient nourishment

nourishment and he considered that the direct origin was toxic, in a way analogous to that in horses viz. from fatigue products which destroy the blood corpuscles.

(65)

Kast published a case in which the attacks occurred only by strong muscular efforts, and he suggests that a fatigue product is the cause.

Many authors have expressed similar views to those just quoted.

Prior made a chemical examination of the urine in his case published in 1888 and found that the uric acid, phosphoric acid, sulphuric acid and urea excretions were diminished during attacks.

Fleischer found no change in the P_2O_5 excretion and as he found the T.N. ^{*} lower, the P_2O_5 relative to T.N. was consequently increased. (loc.cit)

(66)

Kobler and Obermeyer found lessening of the total nitrogen and phosphoric acid excretion. The results were indefinite regarding the ratio of uric acid to urea, but it was most often decreased. The relative P_2O_5 to T.N. was decreased. They considered it a curious fact that there was no increase of chlorides when so much destruction of cells/

* T.N. = Total Nitrogen.

cells was taking place. The urine had not the characters of a fever urine although fever occurred at the paroxysms. They considered that substances were held back for the formation of new cells.

Hayem (page 633) insists that the primary change results from the action of a toxic substance upon the blood as the result of which the haemoglobin is transformed into methaemoglobin. He considers that some substances of this kind have the power to form themselves in our tissues, and he (67) recalls that Kowalewsky has demonstrated that alloxanthin has this property. Hayem more definitely indicated at another time that he believed the toxic substance is formed in the kidney. (Gazette Hebdomadaire 1895 p.283, 284 and 296.)

Fraser found that the urea excreted during the 8 hours of a paroxysm was very much less than that of the corresponding period of the succeeding day. (loc.cit page 336)

(68)
Lockhart Gillespie found an increase on the day of attack.

Urinary Haemoglobinuria i.e. haematuria which becomes converted into haemoglobinuria in the tubules or pelvis of the kidney, in the ureter, or bladder, or after excretion, does not directly concern us in the consideration of the Pathology of Paroxysmal Haemoglobinuria, of which disease nephritis is not a constant or necessary condition. But consideration of the pathology of this form of Haemoglobinuria (to which unfortunately the name "Paroxysmal Haemoglobinuria" is not inapplicable,) if it were sufficiently understood, would serve to show what chemical condition of the urine favoured the solution of haemoglobin.

Such information would be a useful nucleus of fact upon which to proceed to the chemical examination of the blood in paroxysmal haemoglobinuria a frigore. Unfortunately not very much is definitely known regarding the causations of this condition.

Camus (loc.cit) has summarised the work on this subject and has himself done much to extend our knowledge. The concentration of the urine in chlorides is an important matter. The globules may also be destroyed by the acids of the urine especially hippuric acid. Acid phosphate of Soda does not appear to be present in sufficient quantity in the urine to produce any solvent action. The same remark applies to uric acid and sodium urate.

The colouring matters of the urine are strongly toxic. Carbonate of Ammonia and creatin can cause destruction of the red cells.

(pages 80 - 86)

Camus found that the haemolysis of rabbits corpuscles by pathological urines was distinctly attenuated by heating the urines to 56° C., and he asks if this haemolysis ^{has} not been due to the action of alexines i.e. complement.

Camus showed that in certain cases of haemorrhagic nephritis, the haemoglobinuria was of urinary origin even when no hypotonic state of the urine existed. The solution was therefore due to some other factor not ascertained. He also showed that in two consecutive micturitions there might be haemoglobinuria in one and haematuria in the other, or a mixed condition might be present in the urine. These latter changes appeared to depend on the tonicity* of the urine. Camus was able to transform a haemoglobinuria of urinary origin into a haematuria by increasing the tonicity of the urine. He succeeded in doing so by the administration of Sod. Chloride. Conversely he was able to change a haematuria into a haemoglobinuria by giving a very fluid diet poor in salt. Cases of haemoglobinuria of urinary origin can thus be distinguished.

If the solution of the cells is due to urinary globucidal substances or to the hypotonic state of the/

* the saline concentration.

the urine then by increasing the tonicity of the urine the haemoglobinuria will be transformed to an ordinary haematuria. In general the globucidal substances become less powerful as the medium is rendered more concentrated (pages 86 - 98). Camus does not say, but I infer, that if solution of the cells is due to a toxine in the blood serum, then the administration of salt will not hinder the solution as it is difficult to raise the saline concentration of the serum - the salt being speedily excreted.

Such are known facts regarding urinary haemoglobinuria. In the present state of our knowledge it would serve no useful end to speculate as to their application to the haemolysis which results from the action of the serum in paroxysmal haemoglobinuria a frigore. But the systematic examination of the urine in a large number of all kinds of haemoglobinuria would provide data for considering the relation which urinary - haemoglobinuria bears to the haemoglobinuria in which the serum is known to dissolve blood corpuscles. What is to be aimed at is to be able to define the characteristics of haemoglobinuria produced by solution of the haemoglobin,

I. Outside the general circulation.

- (a) in the kidney (Harley) ⁽⁶⁹⁾ etc.
- (b) in the urinary passages (Van Rossem) ⁽⁷⁰⁾ etc.
- (c) in the liver ⁽⁷¹⁾ (Beale) etc.

II. Within the general circulation.

⁽⁷²⁾
(Küssner)

if and when solution does occur in these various situations.

Further it is said that haemoglobinuria results when the liver is called upon to metabolise an excessive quantity of haemoglobin suddenly set free in the serum - Limbeck (13) ⁽⁷³⁾ Rosenbach; and the true relation of the liver to the process of haemoglobinuria can be more accurately determined by investigating the state of nitrogen metabolism as shown by urine analysis.

By such analysis it is possible that our knowledge of the causation may be advanced on the lines suggested by Hayem and Kowalewsky.

Finally such examinations should be conducted in as many cases as possible, if for no other reason than to prove all that is really characteristic of the urine in this disease.

It/

It is well to recall a salient feature of the disease which has been already stated viz. the two important factors which independently may determine a paroxysm in predisposed individuals. One of these is cold and the other is physical effort on the part of the individual. These are apparently very dissimilar, but it is probably correct to assume that each will increase the metabolic processes and the amount of waste products in the blood; in the former circumstance to maintain the the body temperature and in the latter to produce muscular effort.*

It may be further assumed, that each of these conditions will tend to promote an interchange of the constituents of the lymph and blood, so that the products of tissue metabolism will be brought into more intimate relation with the corpuscles. Considering then that the usual precipitating causes of the paroxysm are such as increase metabolic processes it is natural to expect that the paroxysm will be accompanied or followed by an additional excretion of waste products in the urine. That is in any case what we expect in those not affected with paroxysmal haemoglobinuria. In the absence of increased excretion in such individuals, it might be/

* The experiments, already described, indicate that a potential toxine is present at all times in the serum of those cases examined.

be justly assumed that a retention of waste products had occurred.

A further reason for anticipating an increased excretion of waste products follows from the theory above mentioned, that haemoglobin appears in the urine when the liver is overtaxed by an excessive destruction of corpuscles.

This theory, if correct, must surely be supported by evidence that the liver has called on some reserve of metabolic activity before finally capitulating.

Before concluding these general remarks on the subject of metabolism I wish to describe an observation which I have made bearing on the relation of fatigue to the onset of paroxysms.

In the cases of T.S. and J.M. (Sir Thomas Fraser's case) very careful enquiry revealed a fact of considerate importance which so far as I know has not yet been recorded. I learned from them that when they exert themselves they do not become warmed up as was formerly the consequence of their exertions. On the contrary they become colder and colder until they are forced to seek rest and warmth. Any attempt to become warmer by greater exertion proves futile and indeed has the effect of/

the effect of increasing their discomfort from cold and finally of precipitating a paroxysm. In the third case known to me, I was unable to obtain any definite information. This was the case of M.R. a child of 7 years who on account of her illness had been unable to attend school. In consequence of this there was considerable backwardness and to make enquiry of her was entirely out of the question. The parents were very poor. If the weather were fine she was allowed to play about the street. Sometimes she wandered some distance from home and when found, she was generally sitting in a manner indicating an attempt on her part to get warm. This is the voluntary statement of the mother and, if this is an accurate account, does it not appear this is a recognition on the part of the child that by walking she becomes colder?

It is from such considerations as I have stated in the few preceding pages that the condition of the metabolism appears worthy of investigation.

In one of my cases I had frequent opportunities to carry out analysis of the urine under favourable conditions.

The scope of the investigation comprised the estimation of

- (1) The total excretion of Nitrogen in urine.
- (2) The urea Nitrogen of Bohland.
- (3) Non-urea Nitrogen.
- (4) Phosphoric acid.
- (5) Chlorides.
- (6) Toxicity.

The total nitrogen of the urine was estimated by Argutinsky's modification of Kjeldahl's method, the urea nitrogen by Bohlands method, and the non-urea nitrogen by finding the difference between the first two. Two analyses were made of each sample and the results given in the tables are the means of the two estimations. It was necessary to overcome the difficulty arising from the presence of Globulin in the urine and the method employed to separate this was as follows:- A 30% solution of trichloroacetic acid in water was prepared and equal parts of this and urine were titrated together. The fluid was then filtered from the precipitated globulin.

globulin. This process was carried out in all cases whether the urine contained globulin or not. The dilution of the urine was discounted by taking double quantities of the filtrate for the estimation.

The nitrogen in globulin was also ascertained by the Argutinsky method, the filter paper and precipitate being estimated together. The nitrogen value of the filter paper was ascertained and subtracted.

Phosphoric acid was determined by the titration of an estimated quantity of urine with uranium nitrate.

When the patient was in Hospital the urine was collected at regular intervals, in some attacks two hourly, in others four hourly. This methodical collection of the urine was impossible when the patient was at his own home.

The diet of the patient was supervised by the staff nurse who carefully carried out my injunctions. He had stated quantities of bread and milk. Soft foods such as milk puddings were measured in tablespoonfuls. The only variation in the diet from day to day was that fish and chicken were given on alternate days. I found it was exceedingly difficult, and I thought it unnecessary, to give food which/

which would entail special preparing for one patient in a ward, or to have more exact measurements and weighings of the amount carried out.

1st. Paroxysm.

TABLE I.

DAY OF PAROXYSM.

	Amount of urine	Specific Gravity	Reaction acid	Total Nitrogen (less Albumen Nitrogen)	Total Nitrogen per oz. of urine	Albumen Nitrogen	Haemoglobin.
1st 2 hrs.	5 ozs.	1010	-	.298 grms	.06 grms	.061	<u>present</u>
2nd " "	2 "	-	-	.215 "	.107 "	.023	"
3rd " "	2 "	-	-	.296 "	.148 "	.018	"
4th " "	13 "	1002	Neutral	.856 "	.066 "	.031	<u>nil.</u>
5th " "	4 $\frac{2}{3}$ "	1005	Acid	.501 "	.107 "	.016	"
6th " "	same as 5th period.						
7th " "							
8th " "	2 $\frac{1}{3}$ ozs.	1018	Acid	.653 "	.280 "	.012	"
9th " "	same as 8th period.						
10th " "							
11th " "	2 $\frac{1}{3}$ ozs.	-	-	.503 "	.215 "	nil	"
12th " "	same as 11th period.						
Total 24 "	47 "	-	-	6.133 "	.130 "		

Average 2 hrs.

of Haemo-

globinuria

stage.

3 ozs.

.27 "

.09 "

Average
per 2 hrs.

of post

haemoglobin-

uria stage.

4 $\frac{1}{4}$ "

.59 "

.14 "

DAY FOLLOWING PAROXYSM.

Total 24 hrs. 82 "

14.172 "

.17 "

Average
per 2 " 6 $\frac{4}{5}$ "

14.181 "

-

2nd. PAROXYSM.TABLE II.DAY OF PAROXYSM.

		Amount of urine	Specific Gravity	Reaction	2 hrly T.N. * (less Alb.N)	T.N. per oz. of urine	Albumin .N.	Haemo- globin.
Stage I	2 hrly	11.3 oz.	1010	Acid	1.326 grms.	.117 grms.	.113	present
	5½ hours							
Stage II.	"	4.4 "	1010	"	.770 "	.175 "	.034	trace
	5½ hours							
Stage III	"	1.5 "	1022	"	.538 "	.349 "	nil	nil
	13 hours.							
24 hours	Total	53 "			<u>9.265</u> "	.175 "		
Average 2 hrly of haemoglobin- uria stage.		7.8 "			<u>1.048</u> "	<u>.134</u> "		
Average 2 hrly of posthaemo- globinuria stage.		1.5 "			<u>.538</u> "	<u>.349</u> "		

DAY FOLLOWING PAROXYSM.

Total 24 hours	68 "				<u>9.634</u> grms	<u>.142</u> grms		
Average 2 hrly.	5.7 "				.803 "			

* T.N. = Total Nitrogen.

5th PAROXYSM.TABLE III.DAYS PREVIOUS TO PAROXYSM.

		Amount of urine.	Specific gravity	Reaction	T.N. (less Alb.N)	T.N. per oz of urine.	Haemo- globin.
2 days before	2 hourly rate	3.9 ozs	1016	Acid	.842 grms.	.216	Nil
1 day before	" "	" 2.4 "			.751 "	.313	"
Mean of 2 days	" "	" 3.1 "			.796 "	.264	"

DAY OF PAROXYSM.

2a.m. - 6a.m.	"	"	"	(urine lost)	(cold bath at 5.30am)				
6a.m. -10a.m.	"	"	"	5.3 ozs	1015	Acid	.718	"	.135 present
10a.m. - 2p.m.	"	"	"	4.2 "	1012	"	.675	"	.160 "
2p.m. - 6p.m.	"	"	"	6.0 "	1010	"	.934	"	.155 nil
6p.m. -10p.m.	"	"	"	5.7 "	1012	"	.994	"	.174 "
10p.m. - 2a.m.	"	"	"	3.0 "	1015	"	.781	"	.260 "
Haemoglobinuria									
Stage.	"	"	"	4.8 "			<u>.696</u>	"	<u>.146</u>
Post haemo-									
globinuria									
stage.	"	"	"	4.9 "			<u>.903</u>	"	<u>.184</u>

DAYS FOLLOWING PAROXYSM.

1 day after	" "	" 2.3 "			.853 "	.370	"
2 days after	" "	" 4.8 "	1022		1.239 "	.258	"
Mean of 2 days	" "	" 3.5 "			1.046 "	.298	

The paroxysms occurred when the patient was in Hospital.

They were all precipitated by giving a cold bath. From the preceding tables it may be learned that there is nothing characteristic to note regarding the rate of urine excretion during the attacks. This may be greater or less than the rate of the immediately following period of the same day. And the rate on the day of the attack may also be greater or less than the days preceding and following.

Fraser found that the greatest discharge of urine occurred in the eight hours immediately following an attack. (l.c. page 331). This was most commonly so in my cases also.

Depending on this variation, the rate of nitrogenous excretion during the paroxysm also varied, but not to such an extent as to raise the daily total to that registered on the following day, even when the flow was greatest on the day of attack.

The chief fact elicited was that the total nitrogen per ounce of urine was always less during the paroxysms than during the preceding and following days or the remaining hours of the same day.

I doubt if even this point can be considered a characteristic of the attack as the water and the nitrogenous/

nitrogenous matter of the urine are excreted by different processes and at different parts of the kidney. It is probable that the excretion of waste products is hindered by the large excretion of haemoglobin and albumin.

Some authors have even attributed to this retention (?) the primary general symptoms, but this theory probably reverses the order of events. The true state of the metabolism however is possibly obscured by some degree of retention consequent on the elimination of large quantities of haemoglobin and albumin

The relative proportions in which nitrogenous matter is excreted as urea or as less completely metabolised nitrogen has some importance for the following reason.

Assuming the presence of a toxine circulating in the blood, information regarding the distribution of nitrogen excretion will show if the activity of the toxine causes interference with the functions of the liver.

Drs/

Drs. Noel Paton, Craufurd Dunlop and Ivison

(74)

Macadam have already investigated the action of

the diphtheria toxine on the nitrogenous and other

excretions of the dog. Dr. Noel Paton and I (75) have

in a similar way shown that the administration of

such toxic substances as alcohol, coal gas,

sulphonal etc. distinctly interfere with the

elaboration of waste nitrogen into urea in the dog.

In regard to the urea-producing function of the

(76)

liver Von Noorden has stated that during fever

the proportion of ammonia to urea rises very

markedly, and as ammonia is a forerunner of urea,

this must shew a diminished power of the liver to

convert ammonia.

(77)

Dr. Noel Paton concluded that this

modification of the metabolism was the result of

the toxic action of the products of micro-organisms

(78)

and Richard May has published experiments

confirmatory of Noel Paton's conclusions.

TABLE IV.

5th PAROXYSM.

		T.N. per 2 hours	Non Urea N per 2 hours	Urea N per 2 hours	Urea N% of T.N.	P ₂ O ₅ per 2 hours	P ₂ O ₅ per 100 N.		
48 hours preceding attack.		.796 grms	<u>.131</u> grms	.665 grms	<u>83.5</u>	.089 grms	11.5		
Day* of Attack	6-10 a.m.	.718 "	<u>.007</u> "	.711 "	<u>99.6</u>	.148 "	20.5	} Haemo- globin- uria.	
	10a.m.-2p.m.	.675 "	.041 "	.634 "	94	<u>.026</u> "	<u>3.8</u>		
	2p.m.-6p.m.	.934 "	.084 "	.85 "	91	.056 "	6		
	6 - 10 p.m.	.994 "	.025 "	.969 "	97.4	.177 "	17.8		
	10p.m.-2a.m.	.781 "	.042 "	.739 "	94.5	.133 "	17		
48 hours following attack.		1.046 "	<u>.146</u> "	.900 "	<u>86.4</u>	<u>.208</u> "	19		

*
As indicated in Table the urine passed from
2 a.m. - 6 a.m. was lost. This urine did not
belong to the attack as the cold bath was
given at 5.30 a.m.

I had previously made two examinations of urine (obtained while the patient was living at home) to determine the distribution of Nitrogen in the urine.

3rd PAROXYSM.

	T.N.2 hrly.	Non Urea N.	Urea N.	Urea N% of T.N.
Day of illness	.057 grms.	.002 grms.	.055 grms	<u>96.5</u>
Following day	.264 "	.056 "	.208 "	<u>78.7</u>

On the next occasion the urine was only obtained ^{the} on the day of attack but analysis indicates the same characteristic as in the preceding two tables. The patient states that no urine was lost. There was a relapse within the 24 hours as indicated in the table. The amount of urine was 600 c.c.

4th PAROXYSM.

Sample.	T.N.2 hrly	Non Urea N. 2 hourly	Urea N 2 hrly.	Urea N% of T.N.
of 1st 3 hours (port colour)	.274 grms	.023 grms	.251grms	91.6
of 2nd 3 hours (dark sherry)	.252 "	.002 "	.250 "	<u>99.2</u>
of next 10 hrs (much paler but containing Hb.)	.140 "	.014 "	.126 "	90
of remaining 8 hrs.(recurrence)	.208 "	.017 "	.191 "	91.8

The interest of these results is concerned firstly with the distribution of Nitrogen in the urine. There is a very decided departure from the normal in this respect.

Table IV. indicates that during the 48 hours preceding an attack the ratio of Urea Nitrogen to Total Nitrogen was normal viz 83.5%. But with the onset of the paroxysm a sudden and very decided change occurs, as Nitrogen is now excreted almost entirely as urea 99.6%.

At this time therefore only .4% of the total ^{is excreted} nitrogenous matter in the form of non-urea nitrogen which is a striking contrast to the 16.5% of the preceding period.

During the 48 hours subsequent to the day of attack the urea nitrogen percentage had returned almost to normal viz. 86.4%. The non urea nitrogen percentage was therefore 13.6%.

What do these figures indicate?

There are four possible explanations which occur to me:-

- (1) the metabolism of xanthin and uric acid-
^{have been}
-producing tissues may very much reduced.
- (2) It is possible that the partially metabolised
 nitrogenous/

nitrogenous matter is attached or anchored to the globulin and albumin caught by the filter paper after titrating the urine with trichloroacetic acid. We have seen that the corpuscles take up a body from the active serum which is thereafter rendered innocuous to other corpuscles. The constitution of the substance which is taken up and anchored to the cells is unknown, and this second possibility is worthy of further investigation. Professor Robert Muir has found that after haemolysis the Immune body remains attached to the receptors of the cells (l.c. page 447).

- (3) We may have to do with retention of incompletely metabolised nitrogen during the paroxysm; but the figures pertaining to the subsequent days' analyses prove that there is no excess of non-urea nitrogen but merely a return to normal proportions.
- (4) The nitrogenous matter may have undergone a conversion to urea to an extent altogether abnormal .

The results so far as the distribution of nitrogen in the urine are concerned were not what I had anticipated from my previous knowledge of the work of Von Noorden, Noel Paton and Richard May on the metabolism of fever, and of Noel Paton and myself on the action of such toxic substances as Sulphonal alcohol/

alcohol and coal gas on the dog. As I have
already stated, however Kobler and Obermeyer pointed
out many years ago that the urine had not the
characteristics of a fever urine although fever
occurred at the paroxysms.

The phosphorus excretion was investigated in one paroxysm only, but the analysis corroborates the finding of Prior and of Kobler and Obermeyer already referred to. There is a diminution in the excretion of P_2O_5 but a much more striking change is to be seen in the P_2O_5 per 100 N. It is possible that this variation from the normal may not be entirely dependent on the metabolic state, but may to some extent be due to the varying facility with which these substances respectively can pass into the kidney tubules.

(79)

Von Noorden has indicated that the urinary constituents vary enormously in regard to the rate at which they may be excreted by the kidney. He found that phosphates belong to the group of substances excreted with difficulty by the kidney.

It is also possible that the relative proportions of the urinary constituents are further modified by the excretion of haemoglobin and albumin, the/

the more easily excreted substances gaining a readier exit.

As the P_2O_5 was estimated on one occasion only it would be absurd to accept the result which I have obtained as an indication of what is characteristic, but as my figures are in agreement with those of Prior and Kobler and Obermeyer it appears that the P_2O_5 per 100 N during the paroxysm is generally diminished.

Such observations regarding the phosphorus excretion are corroborative ^{evidence} that the metabolism of xanthin and uric acid tissues is reduced during the paroxysm. I have already indicated the possibility of this when discussing the results I had obtained in the investigation of the distribution of the Nitrogen excretion.

Chlorides. Kobler and Obermeyer found that there was a reduced excretion of chlorides. I satisfied myself that there was considerable reduction but I am not in a position to produce exact details.

Pigments. I have frequently made the observation that the normal urinary pigments were conspicuously deficient in the urine of the paroxysm in my cases. This/

This deficiency was made evident after the urine had been treated in the manner already described for separating the haemoglobin preliminary to the urinary analysis. On many occasions the filtrate of haemoglobinuric urine was absolutely colourless and at other times exceedingly pale.

So far as I know this deficiency in the ordinary urinary pigments during the paroxysm has not been previously described, but the observation is in agreement with the idea first suggested by Von Popper (80) that the haemoglobin ceases to be transformed into Bilirubin and passes out in the urine. The deficiency in the excretion of pigments, of nitrogen and of phosphorus, indicate that the liver has not even done its usual amount of work, during the haemoglobinuria period. Therefore the view of Limbeck and others, who consider that haemoglobinuria occurs when the liver is already over taxed is probably wrong. It appears rather that the liver has struck work, and it would probably be incorrect to assume that the passage of haemoglobin accounts for such a retention of other pigments as to render the urine otherwise colourless.

It may be that my observation regarding the deficiency of pigment will come to have some diagnostic/

diagnostic value. I conceive that in a haemoglobin-
 uria of Urinary origin such deficiency of pigment
 may not exist as the metabolism is probably not
 modified in the same manner.

Toxicity

I do not find that any investigation has been
 made in regard to the toxicity of the urine in
 Paroxysmal Haemoglobinuria, although a vast amount of
 work of this kind has been done in France in a
 multitude of diseases. The method that has been
 employed in more recent years was first used by
 Bouchard⁽⁸¹⁾ in 1887. It does not come within the
 scope of the present research either to enter into
 an elaborate criticism of the value of such work or
 to review at length the results that have already
 been obtained. It will be convenient however, to
 preface my own experiments with such a resumé as
 the present investigation requires.

Bouchard made injections of urine into rabbits
 in order to determine the toxic power of the urine
 and the quality of the toxic substance. He found
 that 90 c.c. distilled water killed a rabbit weighing
 1 kilogram. If Sod. Sulphate and Sod. Chloride were
 added to the water so as to make an artificial serum
 the injection of $\frac{1}{2}$ litre was sometimes required
 to cause death. The toxicity of the urine in
 nephritis/

nephritis is less than that of normal urine. When the kidney recovers, one expects to find an excess of toxic material. The toxic dose of normal urine varies from 30 - 60 c.c. to the kilogramme of rabbit. The variations are considerable and become more so with normal urine. By the term "urotoxy" is meant the quantity of toxic substance required to kill 1 kilo. of living tissues. Normally therefore the "urotoxy" is 30 - 60 c.c.'s of ^{physiological} urine. "Urotoxic coefficient" is a term which expresses the number of urotoxies produced in 24 hours per kilogram of the individual supplying the toxic fluid. The average urotoxic coefficient is 0.464. In pathological states it may vary from 0.10 - 2.0. According to Bouchard the potash and colouring matters are the most toxic of the urinary constituents. Urea is very slightly toxic. Distilled water kills quicker than a 4% solution of urea. Uric acid is present in too small amount to be considered the toxic material producing death in the rabbits. The night urine is much less toxic than the day urine and the former is convulsive while the latter has antidotal properties producing narcosis.

The urine after hard exertion in the country is less toxic than the urine of repose and as there is/

is no doubt that hard exertion decidedly increases the mineral matter in the urine there is a large part of the toxicity not attributable to the mineral substances. To a great extent it may be due to organic substances incompletely oxidized whose toxicity diminishes in proportion as oxidation is more completely effected.

Bouchard has demonstrated the plurality of toxins in the urine by separating the solids which are soluble in alcohol from those which are insoluble and thereafter injecting the watery solutions of each of these separately. Those soluble in alcohol produce no convulsions, but sleepiness, coma, diuresis, salivation.

Those insoluble in alcohol produce myosis, convulsions, opisthotonos, vibratory tremor.

On injection of the whole urine there occur additional symptoms exophthalmos, diminution of temperature, nystagmus.

My own experiments were made with the object of determining the degree of toxicity of the urine before, during and after paroxysms. It was also desirable/

desirable to ascertain if the urine of Paroxysmal Haemoglobinuria would produce symptoms which might prove characteristic. The urines which were injected were in most cases analysed as previously recorded.

*

Mattirolo and Tedeschi and other authors believe that in Paroxysmal Haemoglobinuria there is a morbid condition of the kidney which tends to raise the toxic condition of the blood, thus explaining certain cases of Paroxysmal Haemoglobinuria.

Method. The fresh urine was filtered, ** and transfused into the jugular vein of a rabbit of known weight. The urine was allowed to pass into the vein at a uniform rate. ^{animals} Some were done at 5 c.c. per minute and others at 15 c.c. Bouchard considers it necessary to warm the urine or to neutralise it. Precaution must be taken to prevent air getting into the vein.

Nine experiments in all were done.

In three of those, urine was used which had been excreted before the onset of an artificially induced paroxysm. The urine was the mixed urine of 24 hours preceding the 5th paroxysm.

* Loc.cit. page 545 & 546

** through a Pasteur-Chamberland filter.

(1) Rabbit 1210 grammes. Died after the injection of only 14 c.c. urine. Exophthalmus marked increasing with great rapidity. Convulsion before death which took place only 60 seconds after the injection was begun. Unless death was due to some accident, this result proves an unusual degree of toxicity. Urotoxy = 11.5.

(2) Rabbit 530 grammes. Died suddenly when 45 c.c. injected. Urotoxy = 85. The urine was obtained from the same source as in previous experiment.

(3) Rabbit 1265 grammes. Same urine.

Injection begun

12.35 hours

Exophthalmos appeared when 15 cc injected

" marked, pupil contracting 25 cc "

Pupil very small 41 cc 12.37½ "

Breathing irregular 49 cc " 12.38 "

Breathing embarrassed 66 cc "

Convulsions, micturition, defaecation 85 cc "

Violent continuous convulsions 87 cc " 12.41½ "

Death 99 cc " 12.42½ "

Urotoxy 78.

Three /

Three experiments were made with urine passed
on the day of a paroxysm.

In two of these the urine was obtained during
 the 4th Paroxysm.

- (1) Rabbit 1165 grammes. 34 c.c. caused no symptoms
 except slight increase in rate of breathing^{occurring} after
 18 c.c. had been injected.
- (2) Rabbit 830 grammes same urine
- | | |
|---|------------------|
| Injection begun | 12.20 hours |
| frequency of
Increased respiration | 20 c.c. 12.25 " |
| Exophthalmos | 54 c.c. 12.30 " |
| " distinct | 76 c.c. 12.34 " |
| Slight convulsions recurring
occasionally | 82 c.c. 12.35 " |
| Micturition | 101 c.c. 12.40 " |
| Slight convulsion | 111 c.c. |
| Pupil dilated | 116 c.c. 12.42 " |
| Exophthalmos very marked | 124 c.c. |
| No further symptoms except
recurring convulsions | 154 c.c. |
| Urotoxy . . 185 + | |

The third experiment was with urine
 obtained during the acme of the 5th Paroxysm.

Rabbit /

Rabbit 1560 grammes.

Injection begun		12 hours
Exophthalmos beginning	17 c.c.	12.1 "
Breathing slower	34 c.c.	12.1 $\frac{1}{2}$ "
Dyspnoea, pupil contracting	73 c.c.	12.4 "
Breathing shallower	115 c.c.	12.6 "
Slight convulsion	127 c.c.	12.8 "
Marked contraction of Pupil	137 c.c.	12.9 "
Exophthalmos more marked	147 c.c.	12.10 "
Strong convulsions, pupils pin point.	157 c.c.	12.11 "
Death (in 11 $\frac{1}{2}$ minutes)	165 c.c.	12.11 $\frac{1}{2}$ "
Urotoxy = 105		

Urine following paroxysm. Three experiments.

- I. The next specimen of urine injected was that excreted after the subsidence of a paroxysm and before the occurrence of a relapse which followed immediately thereafter. (4th Paroxysm)

Rabbit 230 grammes

Injection begun		12.59 hours
pupil contracting	5.5 c.c.	
Slow breathing, exophthalmos mystagmus	14.c.c.	1.2 hours
Exophthalmos very marked	15 c.c.	
Death	22 c.c.	1.3 hours

Urotoxy = 95.

II. The urine injected was excreted in the 24 hours following the day of 5th paroxysm.

Rabbit = 960 grammes.

Injection begun		5.32 $\frac{1}{2}$ hours
Pupil contracting	19 c.c.	
Exophthalmos	24 c.c.	
Convulsions quickly		
becoming very violent	28 c.c.	
Marked exophthalmos, pupil		
dilated, death	32 c.c.	5.34 hours

Urotoxy = 33.

III. Urine voided three days after a severe
paroxysm (1st.) which was artificially induced.
Rabbit 2620 grammes.

No symptoms until injection of

227 c.c. micturition
270 c.c. exophthalmus marked.
583 c.c. convulsions
611 c.c. death
Urotoxy = 233

Table shewing above results together with the
analysis of the urine.

4th PAROXYSM

Before	During Paroxysm	After
Urotoxy -	<u>185</u>	<u>95</u>
Nitrogen -	{ .462 grammes T.N.	{ .254 grammes T.N.
per 100	{ .439 grammes B.N.*	{ .229 grammes B.N.
c.c. urine -	{ .023 grammes	{ .025 grammes
-	non urea N.	non urea N.

5th PAROXYSM.

Urotoxy ^{mean} <u>58</u> (of 3)	<u>105</u>	<u>33</u>
tests		
Urotoxy <u>.51</u>	<u>.34</u>	<u>.52</u>
coeffi-		
N. per	{ 1.0 gram	{ 1.204 grammes T.N.
100 c.c.	T.N.	
urine	{ .704 gram	{ 1.053 grammes B.N.
	B.N.	
	{ .296 gram	{ .151 grammes
	non urea	non urea N.
	N.	
P ₂ O ₅ per	<u>.058</u> grammes	<u>.270</u> grammes.
100 c.c.		
urine		

Experiment III. of urines excreted after a paroxysm is not included in above tables. It stands by itself as it does not belong to the periods referred to in these tables. The urine ^{was} excreted three days after a paroxysm.

As already said the urotoxy was 233. The amount of fluid injected was very large and may have been fatal in the absence of toxic material. The total Nitrogen per 100 c.c. of urine was .448 grammes.

The experiments although few in number indicate a definite decrease in toxicity of the urine voided during the paroxysm. In the fifth paroxysm it is also shown that the toxicity is even greater immediately after the paroxysm than before it.

It is apparent also that the urotoxy does not vary according to the percentage of total nitrogen or urea nitrogen, sometimes the reverse is the case. It does vary in accordance with the non-urea nitrogen and P_2O_5 and consequently one may assume that these account for the toxic symptoms in some greater degree than does urea. The deficient excretion of pigment during the paroxysm is also to be borne in mind.

No estimation of the urinary sulphates was made. If excretion of the toxic substance of this disease proceeds/

proceeds during the paroxysm it probably does so in combination with the haemoglobin and albumin.

The combination which had taken place in the blood of the patient has probably rendered the toxine innocuous. In this connection it should be remembered that the urines were injected into the rabbit after filtration but without further treatment i.e. the haemoglobin was not eliminated. The chemical analysis on the other hand was preceded by the process for separating the albumin and haemoglobin as already described. The toxic

substance may also have been separated during the process of separating the albumin and haemoglobin. This supposition should be considered along with the ascertained diminution of non-urea nitrogenous and phosphorus excretions in the urine of the paroxysm. Such ^{the} suggestions naturally occur but ^{the} only conclusion

that can be drawn from such a small number of experiments is, that information regarding the chemical nature of the toxic substance may probably be gained by analysing the haemoglobin in the urine. It may be on the other hand that the toxic substance is not excreted during the paroxysm but after its occurrence. The ^{excreted} urine immediately after the fifth paroxysm did/

excreted
 did prove more toxic than that preceding it. Still
 this should not deter one from investigating
 the suggestion that the toxic substance may be
 found in combination with the haemoglobin and
 albumin in the urine.

It only remains to be said regarding the
 preceding experiments on rabbits that the mode of
 death and the preceding toxic symptoms were very
 much the same in all cases, except that when the more
 toxic urines were injected death occurred with fewer premonitory
 symptoms and the convulsions were perhaps stronger.

In order to obtain comparative results I
 conducted another series of experiments with mixed
 physiological urines. I also determined the
 relative toxicity respectively of fresh and boiled
 urine and a solution of the ash of the urine.

In this series I connected the carotid artery
 with a manometer and the usual recording apparatus.
 to facilitate
 Tracheotomy was performed the aether administration.

Five experiments were done with the same
 sample and the following are the results in tabular
 form.

I./

	Rabbit's weight	toxic dose of urine	Urotoxy
I.	1300 grammes	fresh urine {	27
II.	1650 "		47.2
III.	1950 "		10.8 ?
IV.	1450 "	boiled 47 c.c.	32.4
V.	1915 "	ash solution 78 c.c.	41.2

The ash was obtained from 200 c.c. urine and was then dissolved in 200 c.c. of tap water. There was a considerable insoluble deposit. In spite of this the solution proved to be no less toxic than the urines and the mode of death was entirely similar.

Another series of experiments with physiological gave the following urine results. ~~X~~ No manometric tracings were taken on this occasion. ~~X~~

Weight of rabbit	Fatal dose of urine	Urotoxy.
2095	190 c.c. boiled urine	90
1650	64 c.c. fresh "	39
1800	125 c.c. " "	69
1300	65 c.c. ash solution	50
1220	75 c.c. " "	61

The process of calcining changes the salts of the alkalies into carbonates. Bouchard considers the/

the carbonates two or three times more toxic than the chlorides, sulphates or phosphates. Hence he says paradoxical results may be obtained, and death being caused by a smaller dose of ash solution than of the urine itself. There is another probable explanation of such results. The antidotal action of one constituent of the urine for another may be eliminated by the destruction of one of these constituents by the process of calcining.

From the two preceding tables it is evident that the urotoxy varies considerably with the same urine but, almost without exception, within normal limits.

These results confirm the statement already made that the toxicity of urine obtained during a paroxysm of haemoglobinuria is decidedly less than that of normal urine.

MUSCULAR HAEMOGLOBINURIA.

Camus was much impressed by the statement of Mackenzie and others that the serum of the paroxysm does not necessarily become haemoglobinaemic. Camus himself had found, as I have already stated, that considerable colouration of the serum is necessary before Globular Haemoglobin becomes excreted in the urine. Considering these facts, he surmised that Haemoglobinuria might result from the solution of haemoglobin contained in some other tissue of the body than the blood. He remembered that muscular fibre contains haemoglobin proper to itself and he therefore made an attempt to produce an experimental "Muscular Haemoglobinuria."

Method. He sacrificed a dog, ^{by bleeding} and injected several litres of saline water into the aorta, the fluid being allowed to escape by the vena cava. When the muscles were thus washed free of blood, he selected a piece of sufficient size and placed it in a tube, which he kept in a freezing mixture. The muscle was then pounded and the juice was thereafter obtained and diluted with distilled water. Finally, to this was added NaCl so that the solution might be/

be rendered isotonic. 15-20 grammes of muscle furnished 80 c.c. of aqueous solution of muscle juice.

By injecting the solution intravenously into a dog, he produced haemoglobinuria with great ease although the plasma remained uncoloured or showed only a trace of colour. By heating the fluid to 58° C. for $\frac{1}{4}$ hour the power to produce haemoglobinuria was not abolished. If the fluid was boiled no haemoglobinuria was produced by the injections, because the haemoglobin thus became coagulated.

He concluded that muscle haemoglobin can pass through the kidney with much greater facility than globular haemoglobin and an intense haemoglobinuria may occur without any macroscopic modification of the colour of the plasma. The colour of the urine is always more intense than the solution injected, but the amount of haemoglobin that can be obtained from the collected urine is always less than that injected, so that the work of concentration occurs in the kidney. Watery solutions, prepared in a similar way, from liver and spleen did not cause haemoglobinuria.

Injection of distilled water into the substance of the muscles of a dog caused fibrillary tremors and

and haemoglobinuria. The plasma became very pale rose coloured. The amount of distilled water used was not sufficient to produce globular haemoglobinuria when injected intraveinously. Intramuscular injection of glycerine also produced haemoglobinuria in doses which by the intraveinuous method were negative.

Direct electric excitation of muscle for 15 minutes did not cause haemoglobinuria. Placing a limb in a freezing mixture of ice and salt water at the temperature of -6°C . for 55 minutes did not cause haemoglobinuria and ^{caused only} doubtful albuminuria. Injection of watery solution of muscular juice which had been deprived of its haemoglobin did not produce haemoglobinuria. Intravenous injection

of a solution prepared from the white muscles of the rabbit did not produce haemoglobinuria.

A similar preparation from the red muscles of the rabbit caused haemoglobinuria. Such experiments prove that -- in the earlier experiments it was the muscular haemoglobin which passed out in the urine and not the globular haemoglobin.

Camus injected 20 c.c. of a solution of muscular juice into a dog weighing 17 kilogrammes and estimated/

estimated the haemoglobin excreted. One and a half hours later he injected the same quantity of the muscle juice solution + 10 c.c. of a very deep coloured solution of globular haemoglobin and again estimated the haemoglobin in the urine. The same quantity of haemoglobin was collected on each occasion so that only muscular haemoglobin was excreted.

in
In all cases, which haemoglobinuria was produced by the direct action of a noxious substance on the muscle, there was contracture and fibrillary contractions in the muscular masses before haemoglobinuria appeared. Neither of these conditions was observed in a chilled muscle or in muscle after excitation by electricity. (Pages 51-67).^{l.c.}

that Lucet
Camus considers has demonstrated clinically and pathologically that Haemoglobinuria of the horse is a disease of the muscles. He further supports this view with a lengthy quotation from a veterinary authority M. Cadiot as follows :-

"Très habituellement c'est pendant le travail que l'affection éclate; l'invasion est soudaine. D'autant plus ardent à la besogne qu'il vient d'avoir un plus long repos, l'animal se montre tout à coup inquiet/

inquiet, en proie à un malaise qui s'accroît à vue d'oeil ou à des coliques: l'allure se ralentit, le coup de collier est moins franc, il y a des frissons, des tremblements, de la sudation localisée à certaines régions ou généralisée; la respiration est accélérée, parfois plaintive et un peu dyspnéique, la physiognomie est anxieuse, les naseaux dilatés, l'oeil brillant; en même temps ou presque aussitôt apparaissent des troubles de la locomotion. Dans la plupart des cas, ces premiers phénomènes de l'accès hémoglobinurique surviennent dix minutes ou un quart d'heure après la sortie de l'écurie; dans d'autres au bout d'une demi-heure à une heure et quelquefois plus tardivement.

Les troubles de la locomotion, l'un des deux grands symptômes de la maladie, sont variables dans leur forme, leur localisation, leur intensité. Tantôt ils consistent en une raideur généralisée ou localisée par le rhumatisme musculaire; tantôt ce sont des phénomènes paralytiques.

Chez certains sujets une grande partie du système musculaire est affectée; chez la plupart des/

des myosites sont localisées superficielles ou profondes. Aucune region n'en est exempte;"

Camus states that atrophy may follow the muscular lesions.

The serum during an attack is so like the normal colour that some authors have stated that it contains no haemoglobin. Camus himself saw serum which had the normal colour but spectroscopically there were two pale bands of oxy-haemoglobin (pages 68 - 70).

Camus insists on the similarity in the symptoms of the disease in the horse and man. The symptomatic disparity lies alone in the muscular phenomena (page 72 & 73).

He considers that his experiments can explain not only those cases of haemoglobinuria in which the serum has been found to have no abnormal depth of colour but also those in which it is coloured. (p.74)

He considers it useless in the present state of our knowledge to explain the ultimate cause of the disease, but he thinks that fatigue has an increased power to produce some muscular lesions which as in the experimental muscular haemoglobin- result in the excretion uria, would of the haemoglobin of the muscle in the urine. Cold does not produce its effect/

effect by direct action on the muscle but by its influence on the nervous system. Shivering and trembling thus ensue and the consequent production of heat by the trembling muscles is a defence of the organism against the cold. Camus thinks that in this way there is an analogy between the haemoglobinuria a frigore and the haemoglobinuria of fatigue. (pages 75 & 76).

Critique

Camus' valuable experimental work on animals introduces an entirely new idea regarding the pathology of haemoglobinuria in so far as he maintains, that the muscles may be the source, from which comes the haemoglobin excreted in the urine. As I have made no experimental investigation directly bearing on this matter I can only say that while Camus' theory may be to some extent correct it is entirely a supposition in so far as the human disease is concerned. Moreover there are three facts which decidedly support the globular theory. Firstly there is a progressive diminution of the red cells during the paroxysm. Secondly shadow cells may be observed in the urine. Thirdly a toxine which can destroy the blood cells, is contained in the blood serum and lymph.

These /

There may be in some or all cases a simultaneous solution of muscular haemoglobin. If so there is the possibility that the solution is effected in a similar way to that which pertains to the globular destruction. I have not yet had the opportunity of testing the truth of this by experiment for the reason that fresh human muscle, which is entirely free from traces of chloroform or antiseptic lotions, is so difficult to obtain.

THE PRODUCTION OF AN ANTITOXIN.

The experiments which I had done in the summer of 1903, gave such definite evidence of the presence of a toxic body in the serum of cases of Paroxysmal Haemoglobinuria, that I resolved at some future date to attempt the production of an antitoxin. In the period which has since intervened, many new facts have been established regarding the mode of action of the toxic body, and the proposed research has thereby been rendered only the more feasible.

Fortunately my past experience satisfied me that I could obtain, by means of cantharides blisters, considerable quantity of the toxic material for injection into animals. I estimated that I might be able to obtain 40 c.c. of serum from the various cases for the final injection. The adequacy of this amount depended to a great extent on the size of the animal selected for the immunisation. It was desirable therefore to select a small animal, whose blood serum was not very toxic towards human blood. The goat was almost certainly too large to become highly immunised by the dose of toxine, which was available. A few experiments, hereafter recorded, finally led me to select the guinea pig. At first (and, as it has proved, also eventually) the exact object of the injections was to obtain an

antibody/

antibody to the amboceptors, which were contained
respectively in the serum of T.S., J.M., and M.R.

(I had not at this time arrived at the conclusion that the disease resulted from the action of an amboceptor, which was identical in all cases of Paroxysmal Haemoglobinuria, but only that there were amboceptors in each case.)

I did not wish to produce an anti-complement or
antibody to the normal substance in the serum, although

I am not certain that there would have been any disadvantage, (perhaps there would have been an advantage) in doing so. In order to economise time and avoid possible difficulties, I therefore proceeded to inject the paroxysmal haemoglobinuria serum after the complement had been previously inactivated
by keeping the serum at a temperature of 56° centi-
grade for half an hour. My reason for doing so was that I supposed, (rightly or wrongly I am unable to say) that thus anti-complement would not be produced. I was, however, convinced by my study of the literature on the Subject of Immunity that it was
not necessary for an injected substance to be toxic
in order to originate the development of an antibody
in the injected animal. I have already quoted from Metalnikoff, Pfeiffer and Friedberger, and Gruber
in proof of this (page 108 & 109).

I need not again quote from these extremely important researches. The work I proposed to do had not for its object the production of the disease in the guinea pig. I intended simply to inject a substance, (Intermediary body) which alone is incapable of dissolving the corpuscles in the human subject, and which possibly may not be able to act as an Intermediary body between the red cells and the complement of the guinea pig. By inactivating the complement I was actually doing as much as possible to prevent the development of the symptoms of the disease in the guinea pig.

I hoped however that the Paroxysmal Haemoglobinuria amboceptors or intermediary bodies, on injection into the guinea pig, might be an antigene, causing the production of an anti-body or amboceptor. An exactly analogous result was obtained by Pfeiffer and Friedberger when they obtained an anti-immune body to the immune body for cholera vibrios. Although the immune body is toxic only for cholera vibrios yet Pfeiffer and Friedberger succeeded in obtaining an antibody by injecting the immune bodies into an animal.

Experiment. A guinea pig was sacrificed by bleeding and the blood^{was} collected in a small beaker under aseptic precautions. This was allowed to stand in the cold until the serum (A) separated, the beaker being covered with a sterile plug. (B) a 5% suspension of my blood corpuscles in oxalate-NaCl solution was also prepared.

The undiluted serum obtained from the coagulated blood was then used as follows :-

.5 cc of A + 1 cc of B = No haemolysis in 3 hours at 37°C.

.1 " " " + 1 " " " = do. do. do.

.2 " " " + 1 " " " = do. do. do.

Commencing haemolysis in 17 hours.

From such results, it appears that the guinea pig is a fairly suitable animal from which to attempt to procure an antitoxic serum.

Possibly the serum of the guinea pig is not always so weakly toxic for human corpuscles as the above experiment indicates, but the following experiment with rabbits' serum, which proved very toxic, led me to^{select} the guinea pig, for the immunising experiments.

Experiment. A rabbit was killed and blood^{was} obtained and allowed to coagulate as in last experiment.

1 c.c./

1 c.c. rabbit's } + 1 c.c. of B. 3 hours at 37°C.
 serum

Marked haemolysis.
 The haemolysis occurred
 in less than $\frac{1}{4}$ hour.

.5 c.c. rabbit's } + 1 c.c. of B. 3 hours at 37°C.
 serum

Marked haemolysis.
 The haemolysis occurred
 in less than $\frac{1}{4}$ hour.

The rabbit therefore was quite unsuitable for the
 purposes I had in view.

Immunising Experiments.

Feb.1st. Blister serum was obtained from T.S. under
 the usual aseptic precautions. The serum
 had a dark straw colour.

Spectroscopically. Two very faint bands
 of haemoglobin.

Feb.2nd. The serum was inactivated by keeping at
 56°C for 30 minutes.

Guinea pig (A) weighing 630 grammes had
 injected 1.26 c.c. = 2 c.c. per kilogramme.

Feb.3rd. Guinea pig apparently quite well. No
 haemoglobinuria.

Guinea pig A 2.5 c.c. ditto = 4 c.c. per
 kilo.

Feb.4th./

Feb. 4th. Guinea pig has remained apparently well.

No haemoglobinuria.

Feb. 15th. Serum of T.S. was obtained by blistering and was inactivated before being injected. Guinea pig A now weighing 655 grammes had 5.24 c.c. injected = 8 c.c. per kilogramme. The animal was placed in a cold cellar for four hours immediately following injection. No unusual symptoms were observable. The urine obtained in cotton wool showed no naked eye evidences of haemoglobin. Spectroscopic examination confirmed this observation.

Feb. 24th. Serum of T.S. obtained by blistering. Inactivated. Guinea pig A now weighing 660 grammes had 10.5 c.c. injected = 16 c.c. per kilogramme. The animal was then placed under the same conditions as after last injection. No abnormal symptoms.

Mar. 4th. Guinea pig A now weighs 645 grammes. I obtained 17 c.c. of interval serum by means of blisters. Two thirds of the 17 c.c. were obtained from T.S. and one third was obtained from J.M. The amount injected was /

was equal to 26 c.c. per kilogramme.

The animal was then put for 15 minutes in a chamber containing a freezing mixture.

The temperature of the chamber was 11°C .

For 5 minutes of the 15 the animal was kept standing amongst the salt and ice which had a temperature of -1.5°C . The animal shivered very slightly.

March 8th. Guinea pig A was sacrificed by bleeding.

The blood was allowed to clot at a temperature of about 14°C . Blood was obtained in the same way from a normal guinea pig. T.S. was blistered.

Three samples of serum were thus obtained on the following morning - one from the immunised guinea pig, one from the normal guinea pig and one from the blister applied to T.S. No haemoglobin bands were present in any of these. A 5% mixture of blood in pot. oxalate - NaCl solution was made from my blood. It was not desirable to employ washed blood corpuscles in the following experiments, as my ultimate object was to ascertain what would be the effect of injecting the immunised guinea pig's serum into the lymph or blood of individuals suffering from Paroxysmal Haemoglobinuria. In this medium there/

EXPERIMENT I.



there would of course be abundance of human complement, and therefore, by retention of the complement in the in vitro experiments, the conditions in vivo would be the better simulated.

EXPERIMENT I.

March 8th.

The following preparations were placed in ice water for $\frac{1}{2}$ hour and then in the incubator.

- (1) .1 c.c. Haemoglobinuria serum + .1 c.c. oxalate-NaCl solution + .1 c.c. 5% normal blood in oxalate-NaCl solution . There was pronounced haemolysis
+ + +
- (2) .1 c.c. Haemoglobinuria serum + .1 c.c. normal guinea pig serum + .1 c.c. 5% normal blood in oxalate NaCl solution. Same amount of haemolysis.
+ + +
- (3) .1 c.c. Haemoglobinuric serum + .1 c.c. inactivated immunised guinea pig's serum + .1 c.c. 5% normal blood in oxalate NaCl solution.

No haemolysis

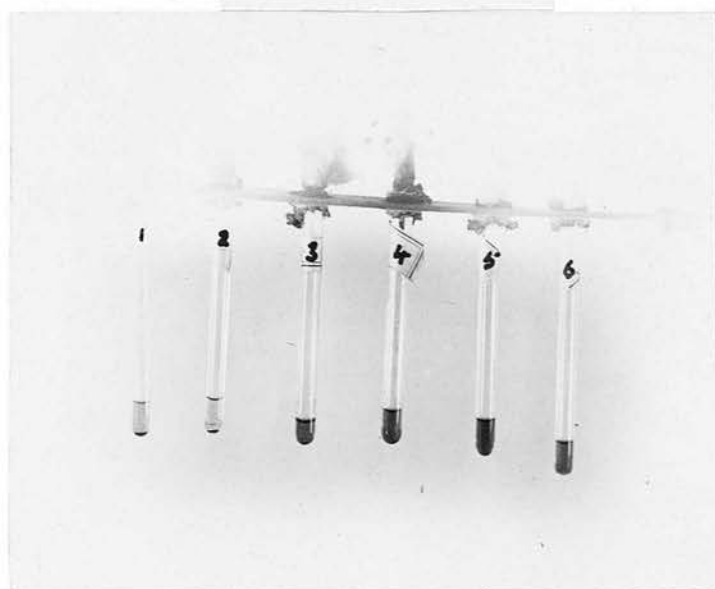
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In 3 the haemoglobinuria serum and the immunised animals serum were allowed to stand in the tubes for 10 minutes before the corpuscles were added. Above observations were made after 4 hours.

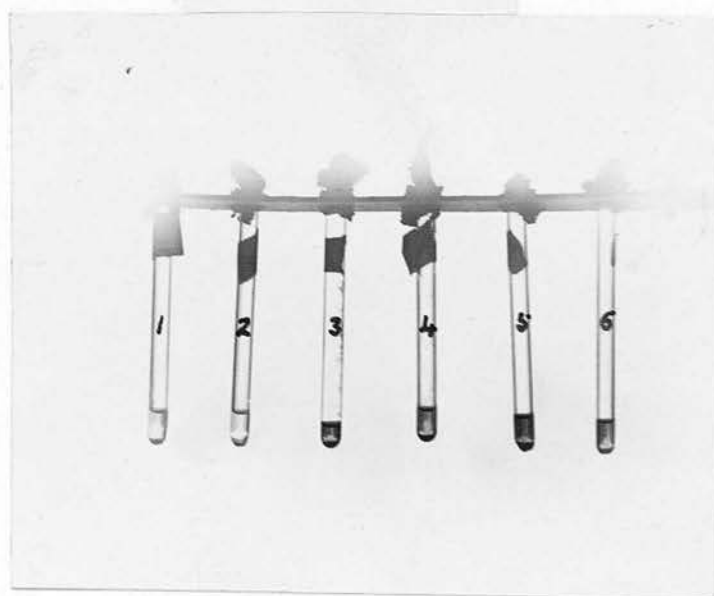
The photograph was taken after 17 hours had elapsed. This experiment gave evidence of the presence/

EXPERIMENT II.

After 4 hours.



After 24 hours.



presence of an anti-amboceptor in the serum of the injected guinea pig, for the amboceptor in the serum of T.S.

EXPERIMENT II

March 9th.

Equal parts of Haemoglobinuria serum and immunised guinea pig serum were mixed together and allowed to stand over night at the room temperature.

The following preparations were then made and placed in ice water for half an hour and then in the incubator for 4 hours.

(1).1 c.c. toxin-antitoxin mixture.

i.e. { .05 c.c. of haemoglobinuric serum
 { .05 c.c. of inactivated serum of
 immunised guinea pig.
 + .05 c.c. of 5% normal blood in
 oxalate - NaCl solution

000

Normal

(2) 5% Blood in oxalate- NaCl solution

000

(3) .05 c.c. toxin (i.e. haemoglobinuria serum)
 + .05 c.c. oxalate- salt solution
 + .05 c.c. normal blood in oxalate-
 NaCl solution

+++

(4) .1 c.c. toxin (i.e. haemoglobinuria serum)
 + .05 c.c. 5% normal blood in oxalate -
 NaCl solution

+++

(5) .1 c.c. normal guinea pig serum
 + .05 c.c. 5% normal blood in oxalate -
 NaCl solution.

+++

(6) .1 c.c. antitoxin (i.e. active serum of
 immunised guinea pig)
 + .05 c.c. 5% normal blood in oxalate
 salt solution

++

The results obtained are confirmatory of Experiment I.

Nos. 1, 2, and 3 are the important tubes.

In/

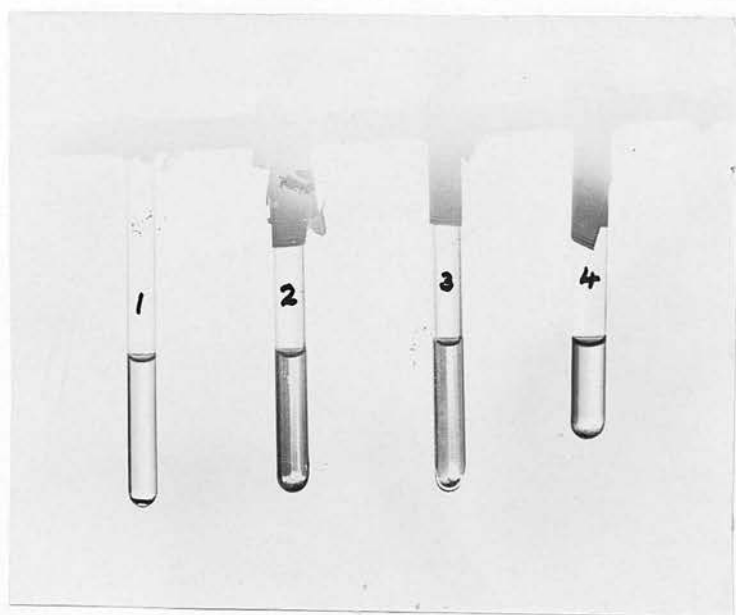
In No.5 it is seen that normal guinea pig's serum dissolves human corpuscles when the mixture is put in ice water for half an hour and then in the incubator. In No.6 the injected guinea pig's serum is also shown to haemolyse normal human blood. When however this antitoxin (?) is mixed with the toxin the haemolytic action of each is neutralized. (No.1)

EXPERIMENT III.

March 16th. In this experiment the same (antitoxic) serum was used. As it was now 8 days old the results prove that the antibody is still potent. The tubes were placed in ice water for $\frac{1}{2}$ hour and thereafter in the incubator for 4 hours.

- | | |
|--|-------|
| (1) .1 c.c. of 5% normal blood in oxalate- NaCl solution | |
| + .1 c.c. oxalate- NaCl solution | 000 |
| (2) .2 c.c. active blister serum of <u>T.S.</u> | |
| + .1 c.c. 5% normal blood in oxalate- NaCl solution. | + + + |
| (3) .2 c.c. active blister serum of <u>M.R.</u> | |
| + .1 c.c. 5% normal blood in oxalate- NaCl solution. | + + + |
| (4) .2 c.c. active normal serum (complement) | |
| + .1 c.c. 5% normal blood in oxalate- NaCl solution. | 000 |
| (5) .2 c.c. active blister serum of <u>T.S.</u> | |
| + .1 c.c. of active normal serum (complement) | |
| + .1 c.c. 5% normal blood in oxalate- NaCl solution | + + + |
| (6) .2 c.c. active blister serum of <u>T.S.</u> | |
| + .1 c.c. of <u>active serum of M.R.</u> | |
| + .1 c.c. 5% normal blood in oxalate- NaCl solution. | + + + |
| (7) .2 c.c. active blister serum of <u>T.S.</u> | |
| + .1 c.c. of <u>immunised guinea pig serum</u> | |
| + .1 c.c. 5% normal blood in oxalate - NaCl solution. | 000 |

EXPERIMENT IV.



The reasons for employing the serum of M.R. in the way I have above indicated are as follows :-

I had made no experiments with the serum since the autumn of 1903. She had improved in health to some extent. I had not seen the urine for many months. I therefore desired to ascertain if her serum had the same toxic properties as formerly. If this were absent I wished then to ascertain if it had anti-toxic properties. The experiments prove that the serum was just as toxic as formerly it had been.

EXPERIMENT IV.

The object of this experiment was to determine if the anti-amboceptor, produced by injecting the serum of T.S. and J.M. into guinea pig A, was also an anti-amboceptor for the amboceptor in the serum of M.R.

March 17th. Same antitoxic (?) serum. The following preparations were kept in ice water 25 minutes and then placed in incubator.

- | | |
|--|-----|
| (1). 2 c.c. 5% normal blood in oxalate -
NaCl solution
+ .2 c.c. oxalate NaCl solution | 000 |
| (2). 2 c.c. active serum of M.R.
+.1 c.c. active normal serum (complement)
+.1 c.c. 5% normal blood in oxalate-
NaCl solution. | +++ |
| (3). 2 c.c. active serum of M.R.
+.1 c.c. inactivated normal serum
+.1 c.c. 5% normal blood in oxalate-
NaCl solution. | ++ |
| (4). 2 c.c. active serum of M.R.
+.1 c.c. immunised guinea pig's inactivated
serum
+.1 c.c. 5% normal blood in oxalate-
NaCl solution. | 000 |

*
Complement
used in
Experiment
III.

The antitoxic serum was therefore antitoxic for the toxine of case M.R. which was not employed in the immunising of Guinea pig A.

This experiment is therefore an extremely important one. It indicates the probability that Paroxysmal Haemoglobinuria is due to the activity of a specific amboceptor, and that the anti-amboceptor which I have obtained is probably the antitoxin for the disease.

Further experiments are being conducted with the object of confirming these conclusions and with the further object of determining what therapeutic value if any may be possessed by serum obtained after injecting guinea pigs in the manner I have described.

Briefly my chief conclusions may be summarised under the following headings.

CLINICAL OBSERVATIONS. (Symptoms etc.)

- (1) The symptoms of Paroxysmal Haemoglobinuria show a general resemblance to those occurring in Malaria and Pernicious Anaemia, the differences being chiefly in degree.
- (2) The occurrence or non-occurrence of Haemoglobinuria in these three diseases is probably to be explained partly by the different degree but also by the different nature of the destructive blood changes characterising them.
- (3) The serum obtained by blistering individuals during paroxysms of Haemoglobinuria usually contains oxy-haemoglobin and sometimes methaemoglobin as my observations show.
- (4) The fibrin formed in serum so obtained (either during paroxysms or at other times) was observed to be of smaller amount than that formed in serum obtained from healthy individuals, and it may be almost entirely absent. May this fact not explain the friability of the blood clot?

clot which has been frequently described as a characteristic of Paroxysmal Haemoglobinuria.

Leucocytes

- 28) (5) Opinions are not agreed as to the relative number of leucocytes circulating in the blood during paroxysms and during intervals, but the most thorough observation already published indicates that there is a slight leucocytosis during the paroxysm.

METABOLISM.

- 19) (6) Analysis of the urine shows that the Total Nitrogen per ounce of urine is diminished during paroxysms, but this may be due to the simultaneous excretion of large quantities of haemoglobin and albumin causing nitrogenous retention.
- 20) (7) The primary general symptoms of Paroxysmal Haemoglobinuria can scarcely be attributed to such retention, as these symptoms precede the changes observed in the urine.
- 21) (8) The ratio of urea nitrogen to Total Nitrogen is much altered during paroxysms so that from the normal (84%) preceding the paroxysm it may increase to 99.6%.

- 22) (9) The explanation of the altered ratio of urea to Total Nitrogen during paroxysms may be that
- (a) the metabolism of xanthin and uric acid-producing tissues is very much reduced:
 - or that
 - (b) the metabolised products of such tissues are excreted in union with the haemoglobin and albumin: or that
 - (c) such products are retained (this is improbable as there is little or no excess excretion of non-urea nitrogen following the paroxysm)
 - or that
 - (d) all nitrogenous matter has undergone more complete metabolism.

Either of the first two possibilities appears to me the most probable explanation of the altered distribution of Nitrogen in the urine.

- 23) (10) The phosphorus excretion appears to be diminished during paroxysms and the ratio to Total Nitrogen is reduced.

- 24) (11) Normal urinary pigments are much reduced in amount during paroxysms and may be entirely absent.

- 25) (12) The activity of the liver is therefore apparently diminished during paroxysms, and the theory that haemoglobinuria results when the liver is called upon to transform an excessive amount of haemoglobin is thus erroneous, unless the liver is unable to do its normal amount of work. Camus' experiments also appear to indicate that the liver cannot hinder the occurrence of haemoglobinuria.

TOXICITY OF THE URINE.

- 26) (13) The toxicity of the urine during paroxysms is distinctly diminished. The degree of toxicity does not vary proportionately with the urea or total Nitrogen present. It varies more in accordance with the non-urea Nitrogen and P_{205} .

- 27) (14) Experiments on the toxicity of the urine of paroxysms when injected into rabbits showed nothing specially characteristic, as regards symptoms.

PATHOLOGY.

- 1) (15) Paroxysmal Haemoglobinuria is a disease resulting from the action of a toxine. My experiments show that not only blood serum but also serum obtained from blisters in individuals affected with Paroxysmal Haemoglobinuria dissolved (in vitro) normal human corpuscles and also /

and also the corpuscles of individuals affected with the disease, provided suitable conditions of temperature exist.

- 2) (16) Under identical conditions of temperature serum of healthy individuals does not dissolve normal corpuscles or corpuscles obtained from those affected with Paroxysmal Haemoglobinuria.
- 3) (17) An unusual degree and form of Phagocytic activity (such as Gruber and Ruziczka consider is characteristic of the union of Intermediary Bodies to red blood corpuscles) has been observed by me in experiments (in vitro) with Paroxysmal Haemoglobinuria serum. This observation indicates the probability that an Intermediary Body is the cause of the haemolysis which I obtained in vitro with Paroxysmal Haemoglobinuria serum.
- 4) (18) My experiments further prove that not only an Intermediary Body which is thermostabile, but also a thermolabile substance - complement - is necessary to produce the haemolysis.
- 5) (19) The changes occurring in the red corpuscles during the progress of solution are similar to those which occur during solution by the haemolytic sera of immunised animals.

- 6) (20) More recent experiments of Donath and Landsteiner and myself appear to confirm the above conclusions, and indicate that the Intermediary Body anchors itself to the red corpuscles when they are together exposed (in vitro) to a temperature lower than that of the body. This union may occur at the ordinary room temperature and also at 0°C . Washed blood corpuscles^{*} do not become destroyed when exposed to a temperature of 0°C .
- 7) (21) The further combination of complement with R.B.C. + I.B. does not occur at 0°C but does so freely at the blood temperature and haemolysis then results.
- 8) (22) Similarly in vivo, atmospheric cold and stasis of the peripheral circulation probably cause a reduction of the temperature of the limbs sufficient to permit the union of the Intermediary Body and red corpuscle. The further union of complement probably occurs most rapidly when the blood returns to the central organs.
- (23) No proof has yet been brought forward that Paroxysmal Haemoglobinuria serum can dissolve muscle haemoglobin.

* Obtained from individuals affected with Paroxysmal Haemoglobinuria

- 9) (24) The chemical nature of the toxine is as yet not ascertained.

ETIOLOGY.

- 10) (25) Cold and fatigue may excite paroxysms in individuals affected with Paroxysmal Haemoglobinuria.
- 11) (26) The probable manner in which cold produces its effect has been already stated. I venture to suggest that the feeling of cold which is produced in affected individuals by prolonged effort throws some light on the manner in which fatigue produces paroxysms.
- 12) (27) Should my observation in reference to the production of the feeling of cold following fatigue prove to be true in all cases of Paroxysmal Haemoglobinuria, cold would then appear to be the essential preliminary to all Paroxysms.
- 13) (28) Is it possible that the feeling of cold may be caused in such individuals by an abnormal fatigue-product which causes vaso motor spasm (Raynaud's symptoms)?
- 14) (29) Consideration of the occurrence of Haemoglobinuria in horses distinctly seems to throw light upon the influence that fatigue has in producing Haemoglobinuria.

- 15) (30) Malaria and Syphilis cannot be excluded as possible sources of the haemolysin substance, but it appears that Paroxysmal Haemoglobinuria may occur without the previous occurrence of either of these diseases.
- 16) (31) Haemoglobinuria may follow a traumatic effusion of blood but phagocytic absorption of the effusion does not appear to be the means by which the haemoglobinuria is produced.
- 17) (32) The Haemoglobinuria in traumatic cases may result from the formation of an Intermediary or Anti-body which acts not only on the effused blood but also on the circulating blood. This supposition has not been experimentally demonstrated, but it appears to be supported by such an experiment as the production of an an auto-toxine for spermatozoa.
- 18) (33) Records do not afford sufficient information to permit the causal relation of trauma to Paroxysmal Haemoglobinuria being accurately determined.

PRODUCTION OF AN ANTITOXIN.

- (34) My experiments appear to show that an antitoxic serum/

*Second
paper*

serum may be produced by injecting an animal (for example the guinea pig) with serum obtained from individuals affected with Paroxysmal Haemoglobinuria.

- (35) In experiments in vitro this serum apparently possesses the property of preventing the production of haemolysis which otherwise would be produced not only by the particular serum used in injecting the guinea pig but also the haemolysis produced by the serum of other cases of the same disease.
- (36) Such experiments in vitro suggest that the toxine may be identical in all cases of Paroxysmal Haemoglobinuria, and that the antitoxic serum might with advantage be employed in the treatment of the disease.

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Rabbit 1300 gms

31st July 1903

Mixed urine of S 1026
fresh

4.2 P.M.

5cc

10cc

60cc

20cc

30cc

35cc

Escalated the amount
of urine passed
Col.

stop

5 min

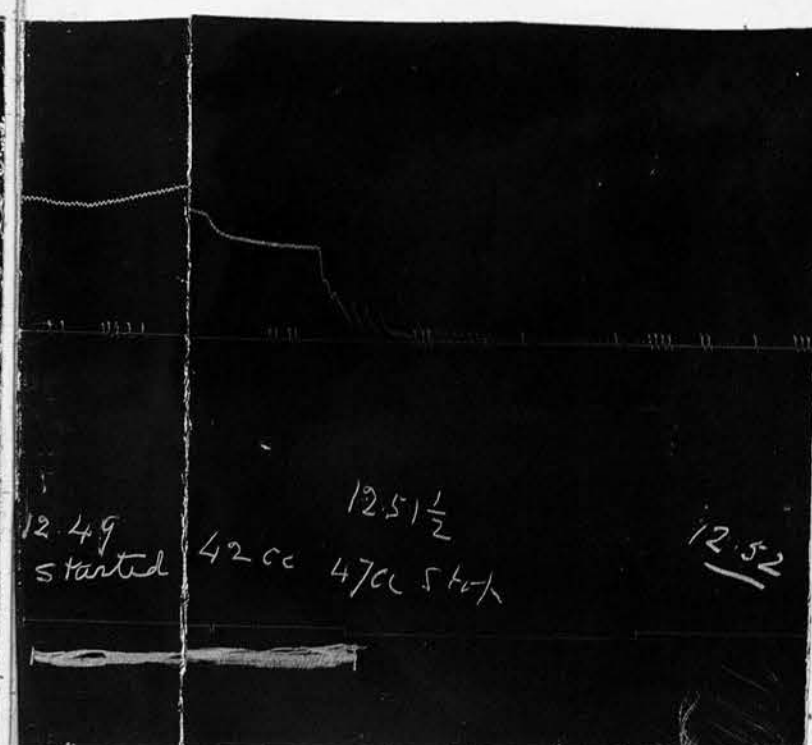
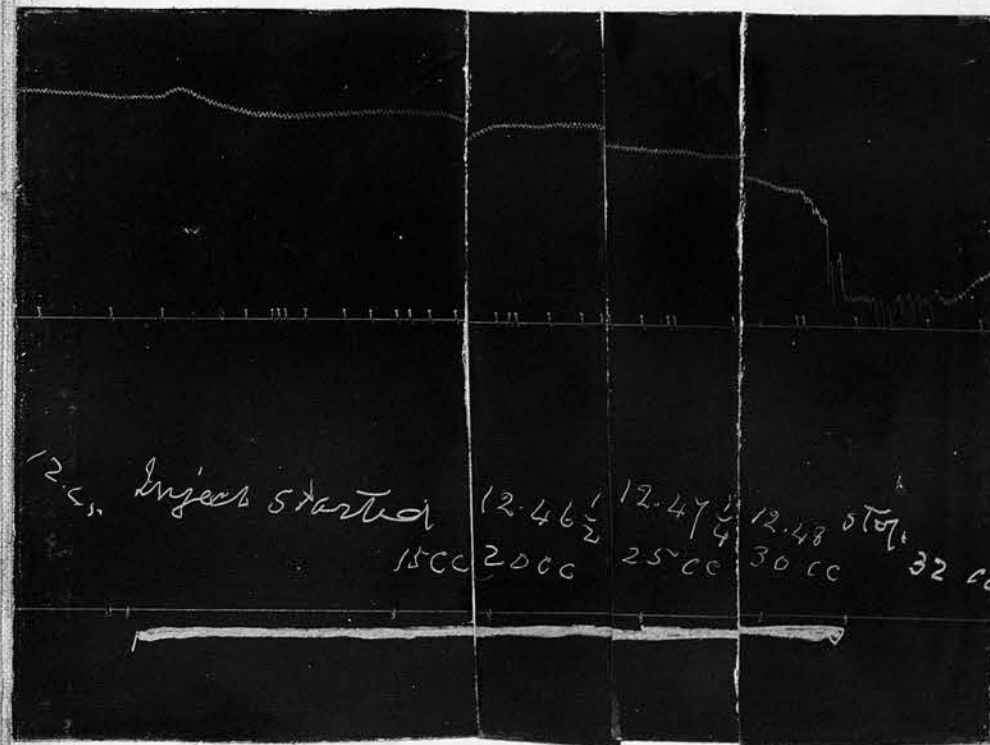
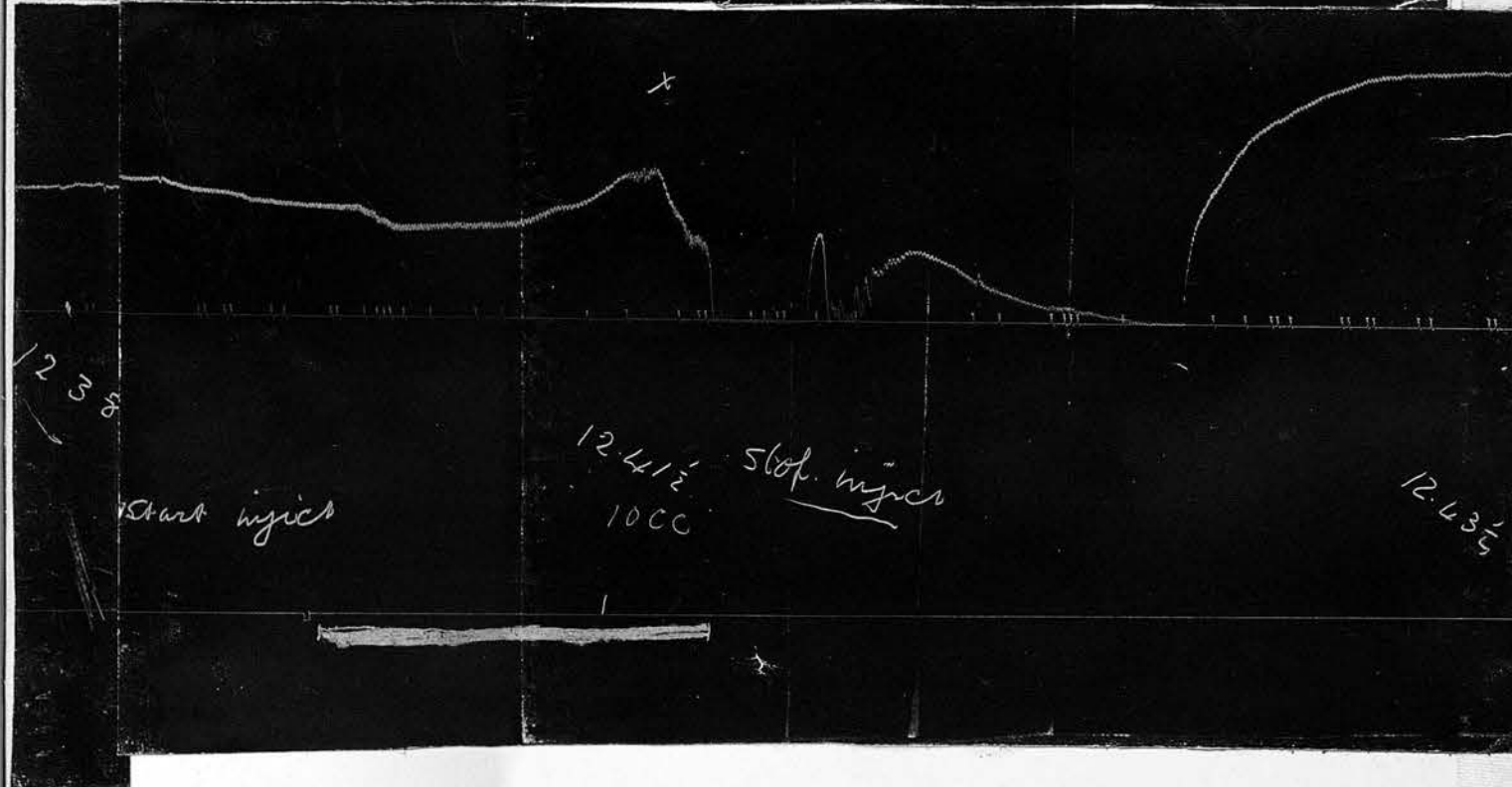
120 sec

3.40 min

Rabbit 1450 gm

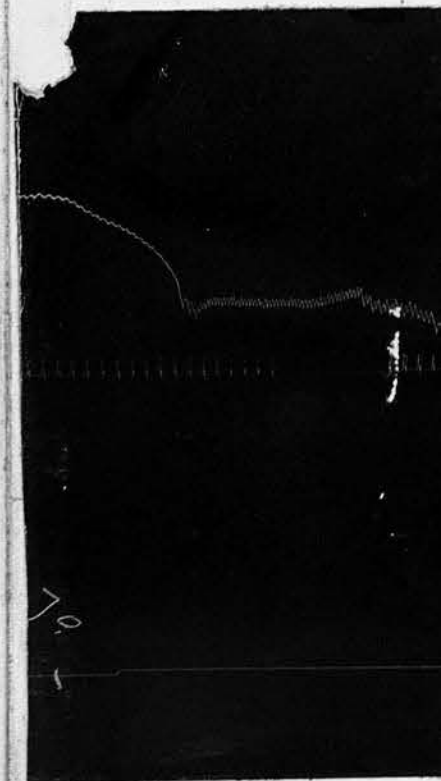
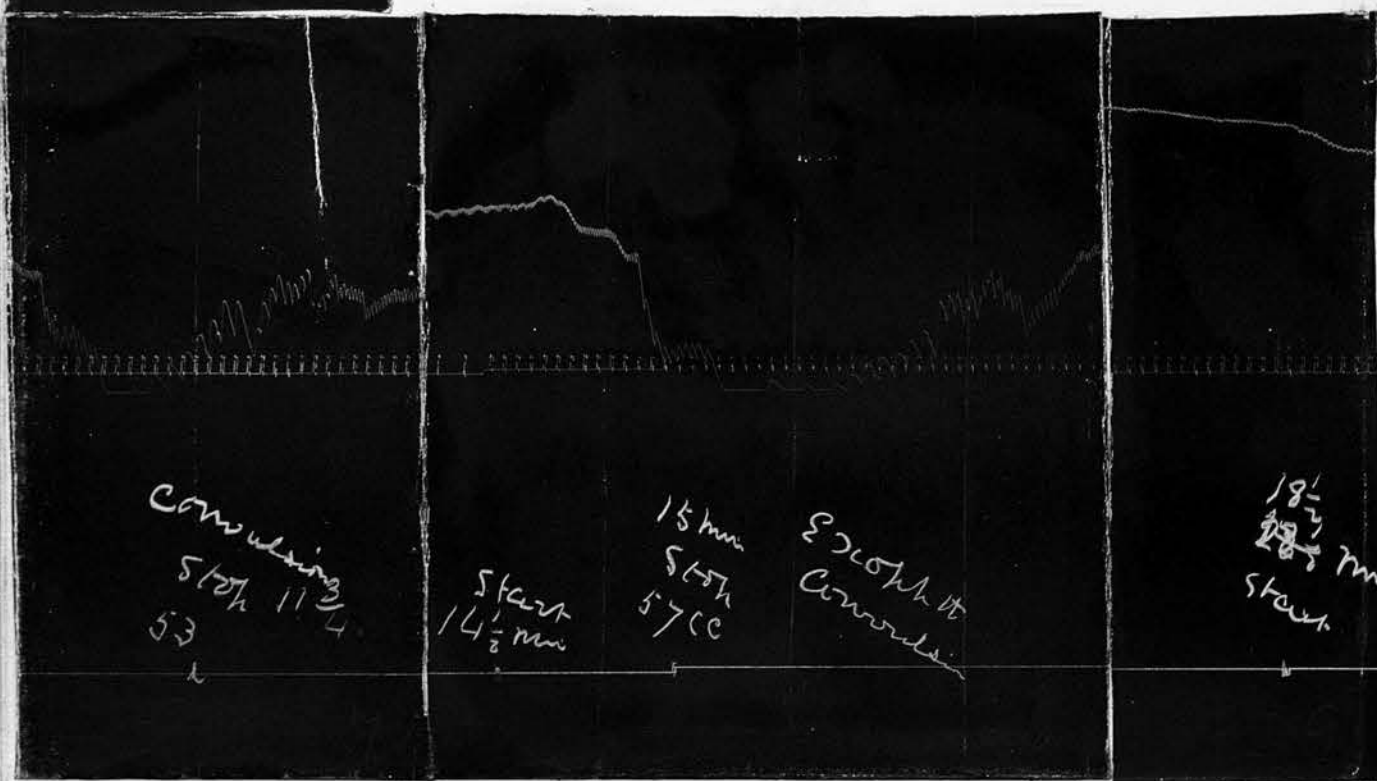
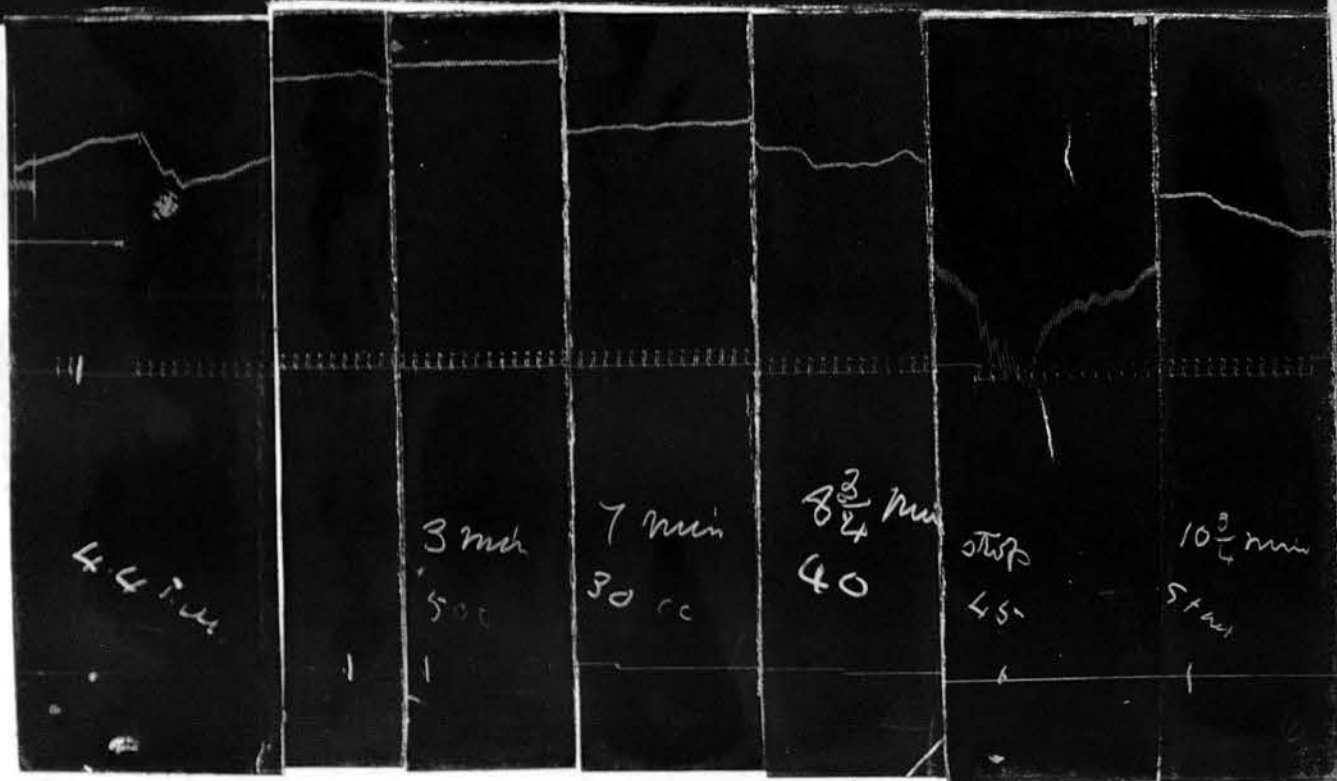
5th Aug.

Winn of 800. 1.2 & 3 (brill)



Each V
Rabbit W-1915 - 6th Aug 1903

Ash of mixed urine of last 9 ads
ash of 200 cc urine in 200 cc tap water



Aug 1903

1st last Pahr

top water

0 3/4 min

Consolidating
5/10 11 1/2
53

Start
14 1/2 min

15 min
5/10
5700

8/10 1/2
Consolidating

18 1/2 min
Start

20 3/4 min
Consolidating

77

79 5/10

22 min
Lifting

74

24

27

29